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Young et al.

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(54) **SUPER-ENHANCERS AND METHODS OF USE THEREOF**

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Related U.S. Application Data

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(60) Provisional application No. 61/718,697, filed on Oct. 25, 2012, provisional application No. 61/799,646, filed on Mar. 15, 2013.

(51) **Int. Cl.**

A01N 25/00	(2006.01)
A61K 47/00	(2006.01)
C12Q 1/68	(2006.01)
C12N 15/85	(2006.01)

(52) **U.S. Cl.**

CPC ***C12Q 1/6804*** (2013.01); ***C12N 15/85*** (2013.01); ***C12Q 1/6869*** (2013.01); ***C12Q 1/6897*** (2013.01); *C12N 2830/00* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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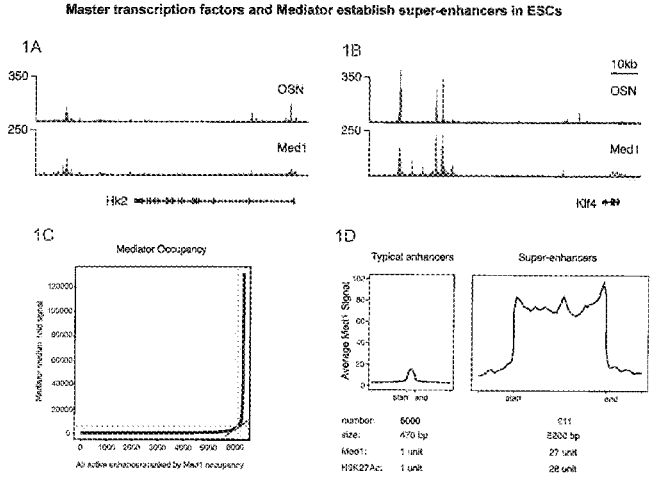
(57) **ABSTRACT**

The present invention relates in some aspects to super-enhancers and related compositions, methods, and agents that are useful for modulating expression of cell type-specific genes that are required for maintenance of cell identity (e.g., embryonic stem cell identity) or maintenance of a disease state (e.g., cancer).

30 Claims, 10 Drawing Sheets



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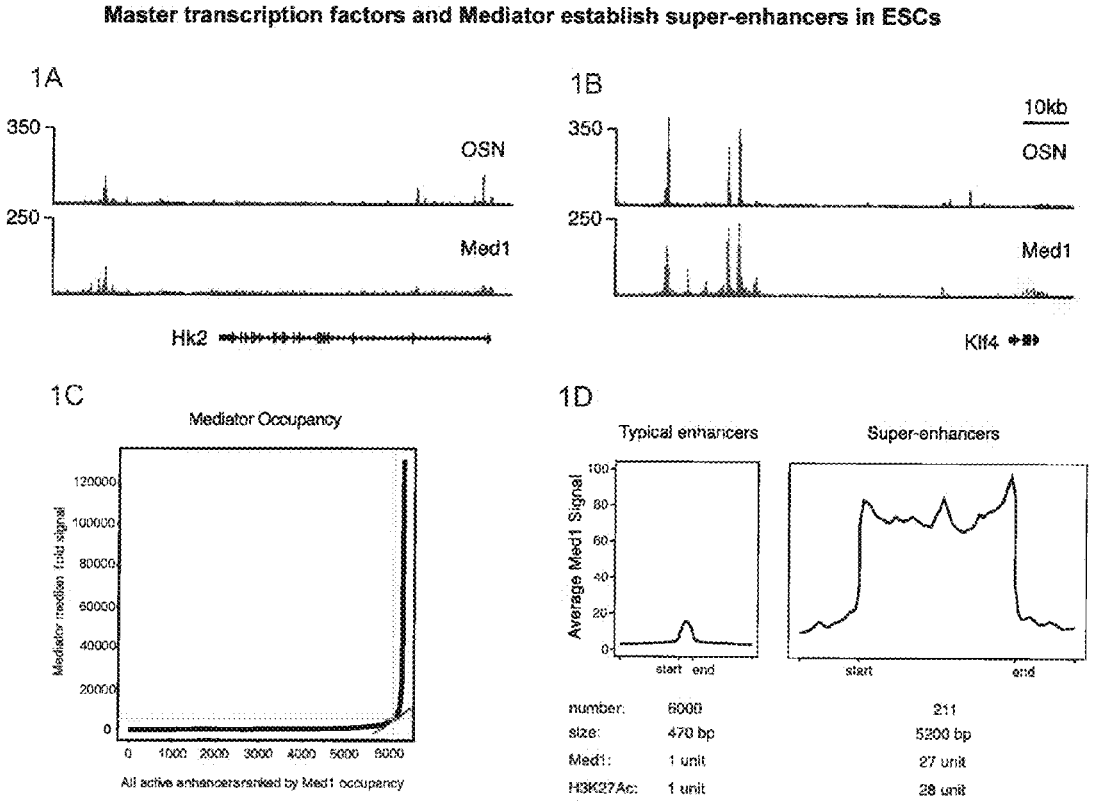
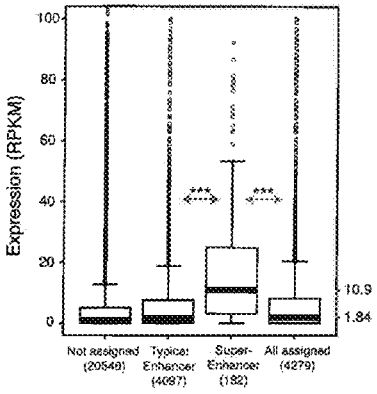
FIGS. 1A, 1B, 1C and 1D



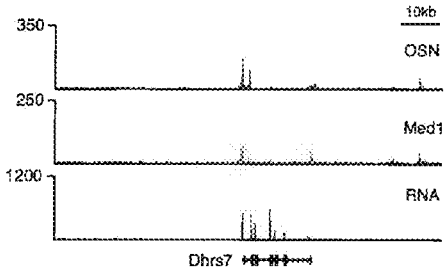
FIGS. 2A, 2B, 2C AND 2D

Super-enhancers are associated with key ESC genes

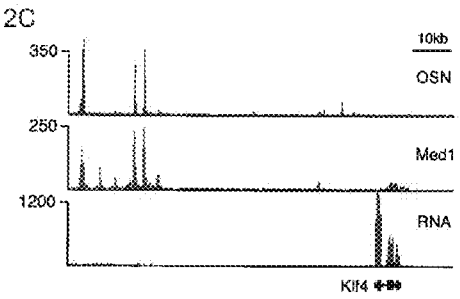
2A



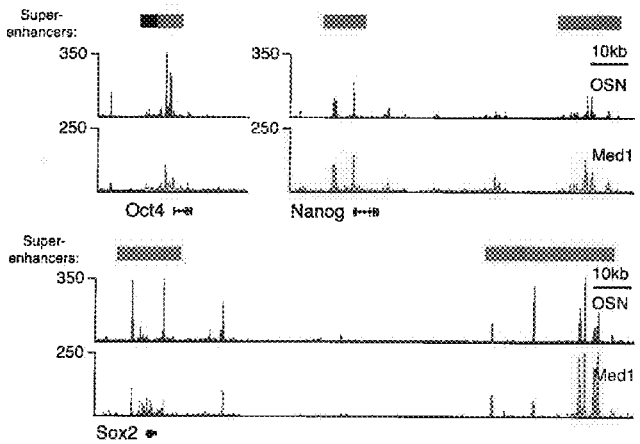
2B



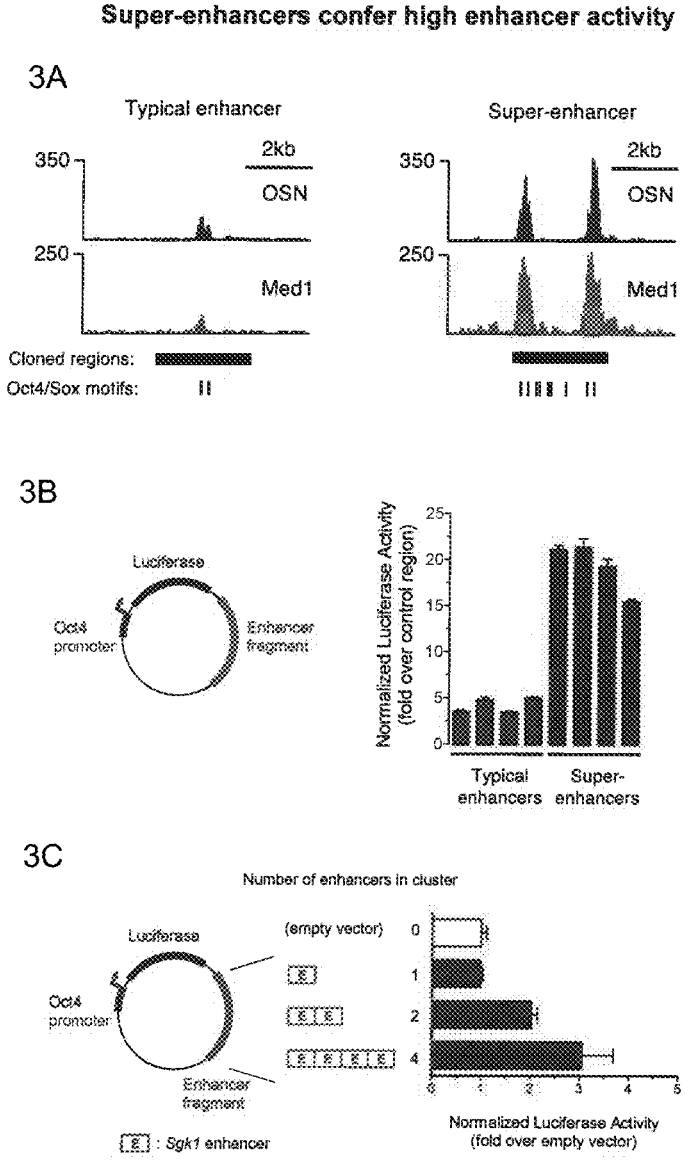
2C



2D



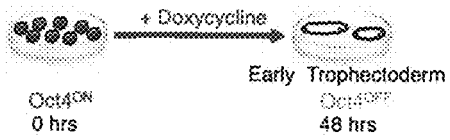
FIGS. 3A, 3B AND 3C



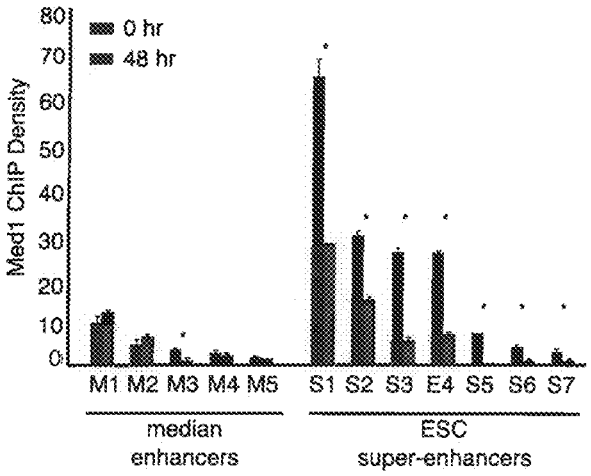
FIGS. 4A AND 4B

Loss of ESC super-enhancers during ESC differentiation

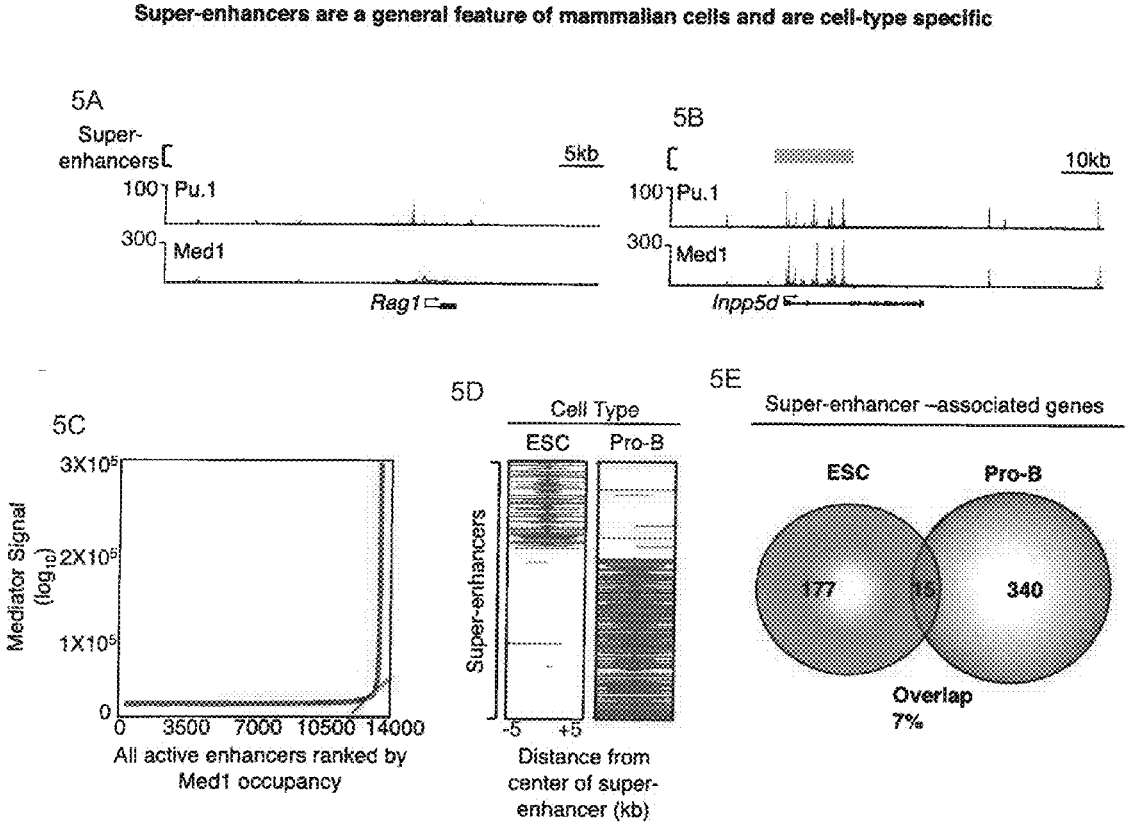
4A



4B



FIGS. 5A, 5B, 5C, 5D and 5E

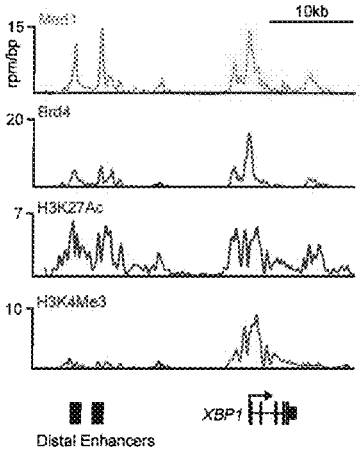


FIGS. 6A, 6B, 6C and 6D

Mediator and BRD4 co-occupy promoters of active genes in multiple myeloma

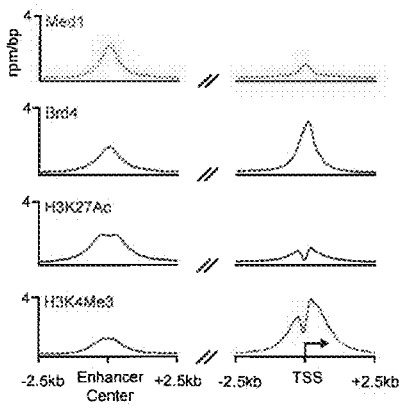
6A

Brd4 occupancy at gene XBP1

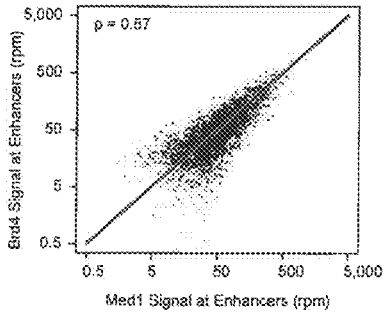


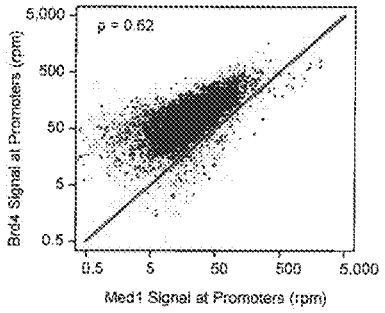
6B

Brd4 occupancy at enhancers and core promoters genome-wide



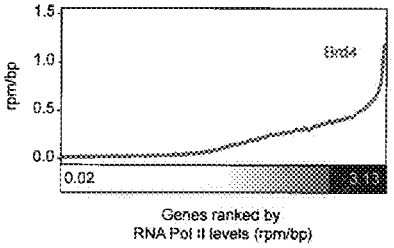
6C Mediator and BRD4 occupancy correlate with one another at both enhancers and transcription start sites





6D

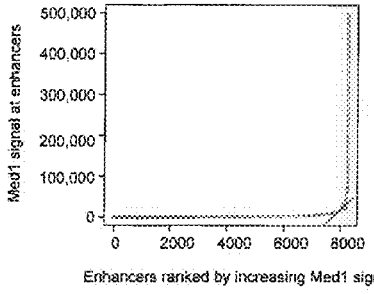
Mediator and BRD4 occupancy at genes correlates with RNAPII levels



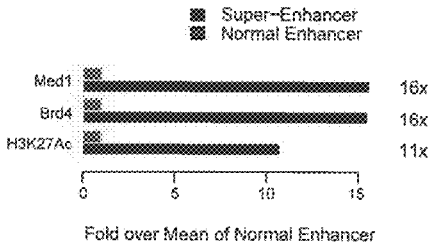
FIGS. 7A, 7B, 7C and 7D

Super-enhancers are associated with key multiple myeloma genes

7A The sizes of enhancers occupied by Mediator show an unusual distribution

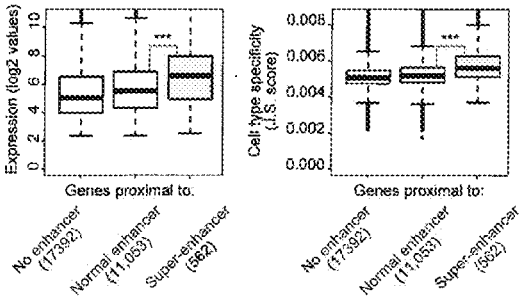


7B Super-enhancers are bound by excpetional levels of BRD4



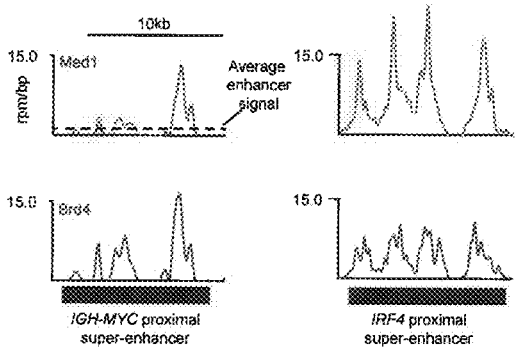
7C

In MM.1S, super-enhancers are associated with highly expressed, cell type specific genes



7D

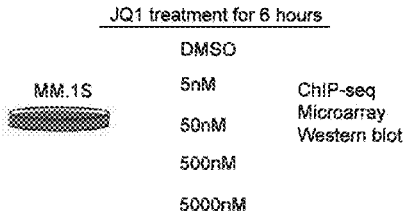
The *IGH-MYC* locus contains a large, 40 kB super-enhancer, occupied by high levels of BRD4 and MED1



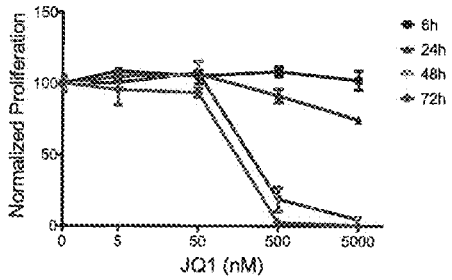
FIGS. 8A, 8B, 8C, 8D, 8E and 8F

BRD4 occupancy at super-enhancers is highly sensitive to bromodomain inhibition

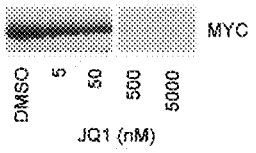
8A Measuring the effects of various concentrations of JQ1 on genome-wide on BRD4 occupancy



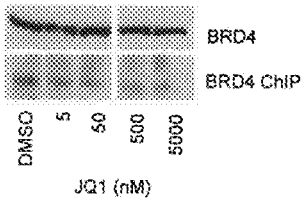
8B . Short-term JQ1 treatment (6 hours) has little effect on MM1.S cell viability



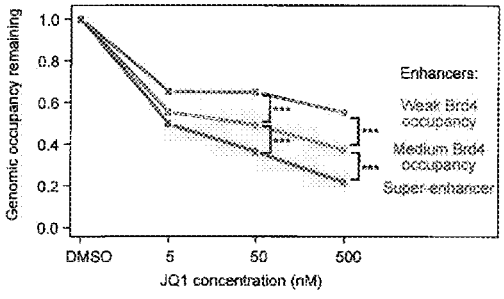
8C c-Myc protein levels are significantly depleted by JQ1 treatment



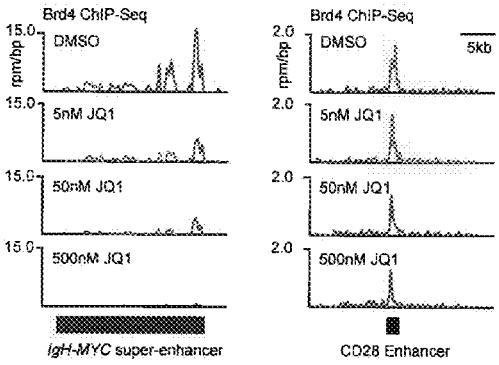
8D JQ1 does not alter BRD4 levels or ChIP-efficency



8E Super-enhancers show a greater loss of BRD4 occupancy when compared to regions with average or low amounts of BRD4



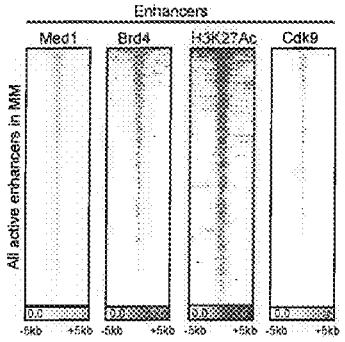
8F The IgH enhancer shows significantly greater loss of BRD4 than regions with lower BRD4 occupancy



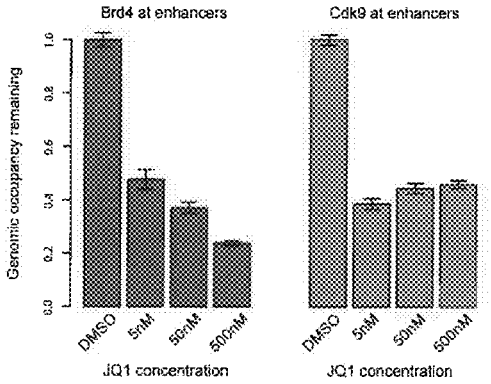
FIGS. 9A, 9B and 9C

Loss of P-TEFb accompanies BRD4 inhibition

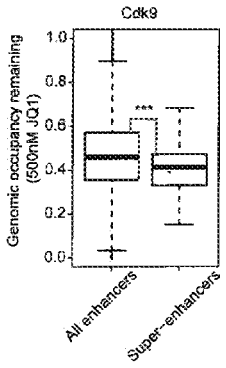
9A P-TEFb generally occupies sites bound by Mediator and BRD4 in MM1.S cells



9B Loss of BRD4 following JQ1 treatment is accompanied by loss of P-TEFb at enhancers



9C P-TEFb is disproportionally lost at super-enhancers

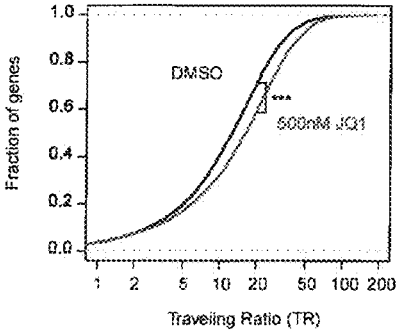


FIGS. 10A, 10B and 10C

JQ1 causes disproportionate loss of transcription at super-enhancer genes

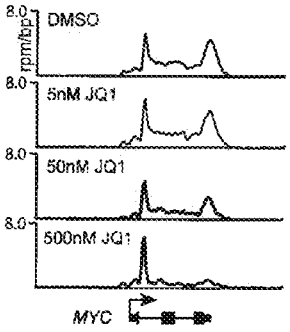
10A

JQ1 treatment causes a global defect in transcription elongation



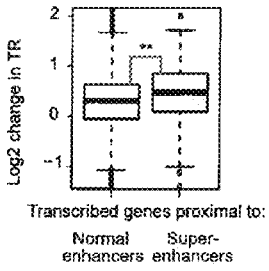
10B

Elongation defect at the *MYC* gene



10C

Genes associated with super-enhancers show a larger increase in TR



SUPER-ENHANCERS AND METHODS OF USE THEREOF

RELATED APPLICATION(S)

This application is a continuation of U.S. application Ser. No. 14/063,337, filed on Oct. 25, 2013, which claims benefit of U.S. Provisional Application Nos. 61/718,697, filed Oct. 25, 2012 and 61/799,646, filed Mar. 15, 2013. The entire teachings of the above application(s) are incorporated herein by reference.

GOVERNMENT SUPPORT

This invention was made with government support under RO1-HG002668 and RO1-CA146445 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Regulatory elements (e.g., transcription factors, cis-acting enhancer elements, transcriptional coactivators and chromatin regulators) activate gene expression programs in cells ranging from embryonic stem cells (ESCs) to tumor cells. Regulatory elements are important for maintenance of cell identity (e.g., ESC identity) and of some disease states (e.g., cancer). The mechanisms underlying how regulatory elements contribute to maintenance of cell identity and of disease state are not entirely understood.

SUMMARY OF THE INVENTION

The present invention relates in some aspects to super-enhancers and related compositions, methods, and agents that are useful for modulating expression of cell type-specific genes that are required for maintenance of cell identity (e.g., embryonic stem cell identity) or maintenance of a disease state (e.g., cancer).

In some aspects, the invention provides an isolated super-enhancer, or functional fragment and/or variant thereof, comprising a genomic region of deoxyribonucleic acid (DNA) that contains at least two enhancers, wherein the genomic region is occupied when present within a cell by more, e.g., 2, 3, 4, 5, 10, or 15 fold more super-enhancer component, e.g., chromatin associated protein, e.g., a transcriptional coactivator, than the average single enhancer within the cell.

A super-enhancer component, as used herein, is a component, typically a protein, that has a higher local concentration, or exhibits a higher occupancy, at a super-enhancer, as opposed to a normal enhancer or an enhancer outside a super-enhancer, and in embodiments, contributes to increased expression of the associated gene.

In an embodiment the super-enhancer comprises all or part of a gene under its control. In an embodiment does not contain a complete associated gene.

In some embodiments the transcriptional coactivator is Mediator. In some embodiments the transcriptional coactivator is Med1.

In some embodiments the genomic region is occupied when present within a cell by more super-enhancer component, e.g., more chromatin regulator than the average single or normal enhancer within the cell.

In some embodiments the chromatin regulator is a BET bromodomain protein. In some embodiments the BET bromodomain protein is BRD4.

In some embodiments the genomic region spans between about 4 kilobases and about 40 kilobases in length.

In some embodiments the genomic region spans sufficient nucleic acid, or the super-enhancer is of sufficient size or structure, such that, when associated with a gene, the gene has substantially greater expression than in the absence of the super-enhancer.

In some embodiments the at least two enhancers are clustered together.

In some embodiments each enhancer comprises a binding site for a cognate transcription factor.

In some embodiments the cognate transcription factor comprises an embryonic stem cell master transcription factor. In some embodiments the embryonic stem cell master transcription factor is selected from the group consisting of Oct4, Sox2, Nanog, Esrrb, Utf1, Klf4, mir-290-295 microRNA gene cluster, Tbx3, Sgk1, and combinations thereof.

In some embodiments a super-enhancer component comprises an enzyme that, adds, detects or reads, or removes a functional group, e.g., a methyl or acetyl group, from a chromatin component, e.g., DNA or histones.

In some embodiments a super-enhancer component comprises an enzyme that alters, reads, or detects the structure of a chromatin component, e.g., DNA or histones, e.g., a DNA methylase or demythylase, a histone methylase or demethylase, or a histone acetylase or de-acetylase that write, read or erase histone marks, e.g., H3K4me1 or H3K27Ac.

In some embodiments a super-enhancer component comprises an enzyme, adds, detects or reads, or removes a functional group, e.g., a methyl or acetyl group, from a chromatin component, e.g., DNA or histones.

In some embodiments the super-enhancer component comprises a protein needed for development into, or maintenance of, a selected cellular state or property, e.g., a state of differentiation, development or disease, e.g., a cancerous state, or the propensity to proliferate or the propensity or the propensity to undergo apoptosis.

In some embodiments the cognate transcription factor comprises an oncogenic transcription factors. In some embodiments the oncogenic transcription factor is selected from the group consisting of c-Myc, IRF4, p53, AP-1, Bcr-Ab1, c-Fos, c-Jun and combinations thereof. In some embodiments the cognate transcription factor comprises a muscle cell transcription factor. In some embodiments the transcription factor is MyoD.

In some embodiments the cognate transcription factor comprises a B cell transcription factor. In some embodiments the transcription factor is Pu.1.

In some embodiments the genomic region is occupied when present within the cell by an order of magnitude more super-enhancer component, e.g., transcriptional coactivator than the average single enhancer within the cell. In some embodiments the order of magnitude is at least about 2-fold. In some embodiments the order of magnitude is at least about 10-fold. In some embodiments the order of magnitude is at least about 15-fold. In some embodiments the order of magnitude is at least about 16-fold.

In some aspects, the invention provides a composition comprising a super-enhancer of the present invention.

In some aspects, the invention provides a nucleic acid construct comprising a super-enhancer, or functional fragment and/or variant thereof, of the present invention. In some embodiments the nucleic acid construct includes a nucleotide sequence encoding a target gene operatively linked to the super-enhancer. In some embodiments the nucleic acid construct includes a reporter construct.

In some aspects, the invention provides a cell transfected with a nucleic acid construct comprising a super-enhancer, or functional fragment and/or variant thereof, operatively linked to a target gene wherein upon transfection of the cell with the nucleic acid construct endogenous transcriptional coactivators and chromatin regulators within the cell co-occupy the enhancers and the active transcription start sites of the target gene to stimulate high levels of expression of the target gene within the cell.

In some embodiments the cell is a mammalian cell. In some embodiments the cell is a human cell. In some embodiments the cell is an embryonic stem cell or embryonic stem cell-like cell. In some embodiments the cell is a muscle cell. In some embodiments the muscle cell is a myotube. In some embodiments the cell is a B cell. In some embodiments the B cell is a Pro-B cell.

In some aspects, the invention provides a method of increasing the level of expression of a target gene in a cell, comprising transfecting a cell under conditions suitable for expression of the target gene with a nucleic acid expression construct comprising a nucleic acid sequence encoding the target gene operatively linked to a super enhancer, or functional fragment and/or variant thereof, wherein upon transfection of the cell endogenous transcriptional coactivators and chromatin regulators within the cell co-occupy enhancers clustered within the super enhancer, or functional fragment and/or variant thereof, and active transcription start sites of the target gene to increase the level of expression of the target gene within the cell. In some embodiments the level of expression of the target gene is increased 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, or more within the cell.

In some aspects the invention provides a kit for increasing the expression of a target gene in a cell, comprising: (a) a nucleic acid construct comprising an artificial super enhancer, or functional fragment and/or variant thereof, operatively linked to the target gene; (b) a population of cells suitable for expression of said target gene; and (c) a reagent for transfecting said population of cells with said nucleic acid construct.

In some aspects the invention provides a method of identifying a super enhancer, or functional fragment and/or variant thereof, in a cell, comprising: (a) identifying a genomic region of DNA within said cell characterized by a cluster of enhancers each of which bind a cognate transcription factor capable of interacting with Mediator to stimulate transcription of the target gene within said cell; (b) measuring in the identified genomic region a level of Mediator; and (c) identifying the genomic region as a super enhancer, or functional fragment and/or variant thereof, if the level of Mediator greater than the level of Mediator occupying the average single enhancer.

In some embodiments the level of Mediator identified in the genomic region is an order of magnitude more than the level of Mediator occupying the average single enhancer. In some embodiments the order of magnitude is at least 2-fold, at least 10-fold, at least 15-fold, at least 16-fold, or more.

In some embodiments the super enhancer, or functional fragment and/or variant thereof, is identified by performing chromatin immunoprecipitation high-throughput sequencing (ChIP-Seq).

In some aspects, the invention provides a method of selectively inhibiting expression of an aberrantly expressed gene comprising disrupting the function of a super-enhancer associated with the aberrantly expressed gene.

In some embodiments the gene is an oncogene. In some embodiments the oncogene is selected from the group consisting of c-MYC and IRF4.

In some embodiments disrupting the function of the super-enhancer comprises contacting said super-enhancer region with an effective amount of an agent that interferes with occupancy of the super-enhancer region by a cognate transcription factor for the gene, a transcriptional coactivator, or a chromatin regulator. In some embodiments the agent is a bromodomain inhibitor. In some embodiments the agent is a BRD4 inhibitor.

In some embodiments the agent is JQ1.

In some embodiments the agent is iBET. In some embodiments the agent interferes with a binding site on the super-enhancer for the cognate transcription factor, interferes with interaction between the cognate transcription factor and a transcriptional coactivator, inhibits the transcription coactivator, or interferes with or inhibits the chromatin regulator.

In some aspects the invention provides a method of treating a proliferative disorder in a patient in need of such treatment, said proliferative disorder characterized by an oncogene-associated super-enhancer occupied by more Mediator or BRD4 than an average single enhancer, comprising administering to the patient an effective amount of an agent that disrupts the function of the oncogene-associated super-enhancer, thereby selectively inhibiting proliferation of the oncogene in the patient.

In some embodiments the proliferative disorder is a hematological malignancy.

In some embodiments the proliferative disorder is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL), Mantle cell lymphoma, B-cell lymphoma, acute lymphoblastic T cell leukemia (T-ALL), acute promyelocytic leukemia, and multiple myeloma.

In some embodiments the agent is a BRD4 inhibitor. In some embodiments the agent is JQ1. In some embodiments the agent is iBET.

In some aspects, the invention provides a method of treating multiple myeloma involving an IGH-MYC locus that results in aberrant expression of oncogene c-Myc, comprising administering to a patient in need of such treatment an effective amount of an agent that decreases occupancy levels of BRD4 and MED1 at a super-enhancer region associated with the IGH-MYC locus, wherein decreased occupancy levels of BRD4 and MED1 at the super-enhancer disrupt function of the super-enhancer thereby decreasing aberrant expression of oncogene c-Myc such that the multiple myeloma is treated. In some embodiments the agent is JQ1 or iBET.

In some aspects the invention provides a method of identifying an agent that disrupts a super-enhancer associated with a gene, e.g., a super-enhancer identified by a method described herein, comprising:

(a) providing a cell or cell-free system having a super-enhancer, or functional fragment and/or variant thereof, associated with a gene, e.g., a gene which is heterologous to one or both of the cell or the super-enhancer, e.g., a reporter construct;

(b) contacting the cell with a test agent, e.g., under conditions suitable for the super-enhancer, or functional fragment and/or variant thereof, to drive high levels of expression of the associated gene; and

(c) measuring the level of expression of the associated gene, e.g., a reporter construct.

In an embodiment decreased expression of the associated gene in the presence of the test agent indicates that the test agent is as an agent that disrupts the super-enhancer associated with the gene.

In an embodiment the method comprises comparing the level of expression with a reference, e.g., a similar cell or cell-free system not contacted with the test agent.

In an embodiment the method comprises confirming disruption of the super-enhancer, or functional fragment and/or variant thereof, e.g., by analysis of the presence of one or more super-enhancer component.

In an embodiment the method is first performed in a cell-free system or a cell preparation, e.g., a cultured cell, and repeated in an animal.

In an embodiment the super-enhancer is associated with a gene that is expressed in a disease state cell, e.g., a cancer cell.

The method, as well as any other method described herein, can include memorializing the results.

In some aspects the invention provides a method of identifying an agent that disrupts a super-enhancer associated with a gene, comprising:

(a) providing a cell or cell-free system having a heterologous super-enhancer, or functional fragment and/or variant thereof, associated with a gene, e.g., a gene which is heterologous to one or both of the cell or the super-enhancer, e.g., a reporter construct;

(b) contacting the cell or cell-free system with a test agent, e.g., under conditions suitable for the super-enhancer, or functional fragment and/or variant thereof, to drive high levels of expression of the associated gene;

(c) and measuring the level of expression of the associated gene, e.g., a reporter construct.

In an embodiment decreased expression of the associated gene in the presence of the test agent indicates that the test agent is as an agent that disrupts the super-enhancer associated with the gene.

In an embodiment the method comprises comparing the level of expression with a reference, e.g., a similar cell or cell-free system not contacted with the test agent.

In an embodiment the method comprises confirming disruption of the super-enhancer, or functional fragment and/or variant thereof, e.g., by analysis of the presence of one or more epigenetic super-enhancer component.

In an embodiment the method is first performed in a cell-free system or a cell preparation, e.g., a cultured cell, and repeated in an animal.

In an embodiment the super-enhancer is associated with a gene that is expressed in a disease state cell, e.g., a cancer cell.

In some aspects the invention provides a method of identifying an agent that disrupts a super-enhancer associated with a gene, comprising: (a) transfecting a cell with a super-enhancer, or functional fragment and/or variant thereof, and the associated gene under conditions suitable for the super-enhancer to drive high levels of expression of the associated gene; (b) contacting the cell with a test agent; (c) and measuring the level of expression of the associated gene, wherein decreased expression of the associated gene in the presence of the test agent indicates that the test agent is as an agent that disrupts the super-enhancer associated with the gene.

In an embodiment the method comprises comparing the level of expression with a reference, e.g., a similar cell not contacted with the test agent. In an embodiment the method comprises confirming disruption of the super-enhancer, or functional fragment and/or variant thereof, e.g., by analysis of the presence of one or more super-enhancer component. In an embodiment the method is first performed in a cell-free system or a cell preparation, e.g., a cultured cell, and repeated in an animal.

In an embodiment the super-enhancer is associated with a gene that is expressed in a disease state cell, e.g., a cancer cell.

In some aspects the invention provides a method of identifying an agent that disrupts a super-enhancer comprising: (a) transfecting a cell with a super-enhancer operably linked to a reporter construct comprising a reporter gene under conditions suitable for the super-enhancer to drive high levels of expression of the reporter gene; (b) contacting the cell with a test agent; (c) and measuring the level of expression of the reporter gene, wherein decreased expression of the reporter gene in the presence of the test agent indicates that the test agent is as an agent that disrupts the super-enhancer.

In some embodiments the super-enhancer is naturally associated with a gene of interest, wherein the gene of interest is optionally a disease-associated gene, optionally an oncogene. In some embodiments expression is measured at least in part by measuring the level of a gene product encoded by the gene or by measuring activity of a gene product encoded by the gene. In some embodiments a gene product is mRNA or polypeptide encoded by the gene.

In some aspects, the invention relates to a method of identifying a super-enhancer, or a gene associated with a super-enhancer, comprising:

cross-linking, e.g., covalently cross-linking, chromatin, such that chromosomal nucleic acid is cross-linked to a super-enhancer component, e.g., a chromatin associated protein, e.g., one or more of a Mediator protein, Med1, Oct4, Sox2, Nanog, or NOS, to form a cross-linked complex;

contacting said cross-linked complex with a ligand having affinity for the super-enhancer component, e.g., an antibody or small molecule with affinity for the super-enhancer component to form a complex between the cross-linked complex and the ligand;

optionally, identifying or sequencing chromosomal nucleic acid in the complex between the cross-linked complex and the ligand, thereby identifying a super-enhancer, or a gene associated with a super-enhancer.

In an embodiment the method comprises fragmenting the chromosomal nucleic acid, e.g., after the step of forming a cross-linked complex, or after forming the complex between the cross-linked complex and the ligand.

In embodiments the method comprises identifying a gene associated with the super-enhancer.

In embodiments the method comprises classifying an enhancer as having a first or second level of occupancy, wherein said first level is higher, e.g., 2, 5, 10, or 100 times higher than the second level.

In some aspects, the invention relates to a method of identifying a super-enhancer, or a gene associated with a super-enhancer, comprising:

identifying sites on a segment of chromosome that are hypersensitive to reaction with an agent, e.g., a nuclease, e.g., a DNase, e.g., DNase I;

identifying or sequencing chromosomal nucleic acid adjacent the sites; thereby identifying a super-enhancer, or a gene associated with a super-enhancer.

In an embodiment the method comprises fragmenting the chromosomal nucleic acid, e.g., after the step of forming a cross-linked complex, or after forming the complex between the cross-linked complex and the ligand.

In embodiments the method comprises identifying a gene associated with the super-enhancer.

In an embodiment, the method comprises confirming, e.g., by sequencing, that a candidate super-enhancer site comprises a plurality of enhancers.

In embodiments the method comprises classifying an enhancer as having a first or second level of occupancy, wherein said first level is higher, e.g., 2, 5, 10, or 100 times higher than the second level.

The practice of the present invention will typically employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant nucleic acid (e.g., DNA) technology, immunology, and RNA interference (RNAi) which are within the skill of the art. Non-limiting descriptions of certain of these techniques are found in the following publications: Ausubel, F., et al., (eds.), *Current Protocols in Molecular Biology, Current Protocols in Immunology, Current Protocols in Protein Science*, and *Current Protocols in Cell Biology*, all John Wiley & Sons, N.Y., edition as of December 2008; Sambrook, Russell, and Sambrook, *Molecular Cloning; A Laboratory Manual,* 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001; Harlow, E. and Lane, D., Antibodies—A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988; Freshney, R. I., "Culture of Animal Cells, A Manual of Basic Technique", 5th ed., John Wiley & Sons, Hoboken, N.J., 2005. Non-limiting information regarding therapeutic agents and human diseases is found in Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th Ed., McGraw Hill, 2005, Katzung, B. (ed.) Basic and Clinical Pharmacology, McGraw-Hill/Appleton & Lange; 10th ed. (2006) or 11th edition (July 2009). Non-limiting information regarding genes and genetic disorders is found in McKusick, V. A.: Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press, 1998 (12th edition) or the more recent online database: Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), as of May 1, 2010, World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/ and in Online Mendelian Inheritance in Animals (OMIA), a database of genes, inherited disorders and traits in animal species (other than human and mouse), at http://omia.angis.org.au/contact.shtml. All patents, patent applications, and other publications (e.g., scientific articles, books, websites, and databases) mentioned herein are incorporated by reference in their entirety. In case of a conflict between the specification and any of the incorporated references, the specification (including any amendments thereof, which may be based on an incorporated reference), shall control. Standard art-accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIGS. **1**A, **1**B, **1**C and **1**D demonstrate that Oct4/Sox2/Nanog define enhancers in ES cells. FIG. **1**A depicts an example enhancer upstream of the Hkt2 gene. FIG. **1**B depicts an example of super-enhancer upstream of the Klf4 gene. FIG. **1**C is a scatter plot showing Mediator occupancy across the ~6,400 ESC enhancers. FIG. **1**D illustrates metagenes of Med1 at typical and super-enhancers in ESCs.

FIGS. **2**A, **2**B, **2**C and **2**D demonstrate that Super-enhancers drive key pluripotency genes. FIG. **2**A demonstrates that super-enhancers drive highly expressed genes. FIG. **2**B depicts an example of typical enhancer-associated gene (with RNA-seq). FIG. **2**C depicts an example of a super-enhancer-associated gene (with RNA-seq). FIG. **2**D illustrates that super-enhancers associate with the ESC master regulators Oct4, Sox2 and Nanog.

FIGS. **3**A, **3**B and **3**C demonstrate that super-enhancers confer high enhancer activity. FIG. **3**A shows OSN and Mediator gene tracks at enhancers near Klf4 (super-enhancer associated gene), and Egln3 (typical enhancer-associated gene), and corresponding DNA binding motifs. FIG. **3**B demonstrates that super-enhancers have high enhancer activity in vitro. 3000 bp genomic fragments were cloned into a luciferase reporter plasmid. Luciferase activity was measured 24 hours post transfection, and was normalized to a co-transfected control plasmid. FIG. **3**C depicts the creation of artificial super-enhancers by clustering. Single enhancers were genetically oligomerized and cloned into luciferase reporters. Luciferase activity was measured 24 hours post transfection, and was normalized to a co-transfected control plasmid.

FIGS. **4**A and **4**B demonstrate rapid loss of ESC super-enhancers and establishment of new super-enhancers during ESC differentiation. FIG. **4**A is a cartoon diagram depicting treatment of ZHBTc4 ESCs with doxycycline leading to loss of Oct4 proteins, loss of ESC state, and formation of early trophectoderm cells. FIG. **4**B illustrates that Mediator is rapidly lost at key ESC super-enhancers compared to median enhancers. Bar graphs of mean normalized Med1 density before and during ESC differentiation at selected ESC super-enhancers and median enhancers. The associated genes were identified based on their proximity to the enhancers. Asterisks denote enhancers displaying at least two-fold reduction in Mediator.

FIGS. **5**A, **5**B, **5**C, **5**D and **5**E demonstrate that super-enhancers are a general feature of mammalian cells and are cell-type specific. FIG. **5**A demonstrates that Pro-B enhancers are associated with the gene Rag1. ChIP-Seq binding profiles (normalized reads/million) for the pro-B transcription factor (Pu.1), and the Mediator coactivator (Med1) at the Rag1 locus in pro-B cells, with the y-axis floor set to 1. Gene model, and previously described enhancer regions are depicted below the binding profiles. FIG. **5**B demonstrates that pro-B super-enhancers are associated with the key pro-B gene Inpp5d. ChIP-Seq binding profiles (normalized reads/million) for the pro-B transcription factor (Pu.1), and the Mediator coactivator (Med1) at the Inpp5d locus in pro-B cells, with the y-axis floor set to 1. Gene model, and previously described enhancer regions are depicted below the binding profiles. FIG. **5**C is a scatter plot of Mediator occupancy across the ~13000 pro-B enhancers. FIG. **5**D demonstrates that master transcription factors (Oct4 for ESCs; Pu.1 for pro-B cells) and Mediator occupy approximately super-enhancer regions that are specific for ESCs and pro-B cells. Density maps of the Mediator coactivator (Med1) in ESCs and pro-B cells. Color scale reflects ChIP-Seq signal in reads per million. FIG. **5**E demonstrates that super-enhancer associated genes display highly cell-type specific patterns of expression. Venn diagram of ESC super-enhancer-associated genes and pro-B super-enhancer-associated genes.

FIGS. **6**A, **6**B, **6**C and **6**D demonstrate that Mediator and BRD4 co-occupy promoters of active genes in multiple myeloma. FIG. **6**A depicts gene tracks of BRD4, MED1, H3K27ac, and H3K4me3 binding at the XBP1 gene in MM.1S multiple myeloma. FIG. **6**B is a meta-gene representation of global BRD4, MED1, H3K27ac, and H3K4me3

occupancy at enhancers and promoters. The top 5,000 active enhancers are defined by MED1 occupancy, and TSS includes all transcriptionally active promoters defined by H3K4me3 and POL2. FIG. **6**C demonstrates that Mediator and BRD4 occupancy correlate with one another at both enhancers and transcription start sites. Scatter plots depicting MED1 and BRD4 aggregate signal +/− 5kb from enhancers and promoters (as defined in **1**B). FIG. **6**D demonstrates that BRD4 occupancy at genes correlates with RNAPII levels.

FIGS. **7**A, **7**B, **7**C and **7**D demonstrate that super-enhancers are associated with key multiple myeloma genes. FIG. **7**A demonstrates that the sizes of enhancers occupied by Mediator show an unusual distribution. FIG. **7**B depicts occupancy of MED1, BRD4, and H3K27ac at super-enhancers compared to normal enhancers. FIG. **7**C demonstrates that super-enhancers are associated with highly expressed, cell type specific genes. FIG. **7**D demonstrates that the IgH-MYC locus and IRF4 contain a large super-enhancers occupied by high levels of BRD4 and MED1.

FIGS. **8**A, **8**B, **8**C, **8**D, **8**E and **8**F demonstrate that BRD4 occupancy at super-enhancers is highly sensitive to bromodomain inhibition. FIG. **8**A depicts measuring the effects of various concentrations of JQ1 on genome-wide on BRD4 occupancy. Schematic depicting the experimental procedure. FIG. **8**B demonstrates that short-term JQ1 treatment (6 hours) has little effect on MM.1 S cell viability. JQ1 sensitivity of MM.1S cells by measurement of ATP levels (CellTiterGlo) after 6 hours of treatment. FIG. **8**C illustrates that c-Myc protein levels are significantly depleted by JQ1 treatment. Western blot of relative c-MYC levels after 6 hours of JQ1 or DMSO treatment. FIG. **8**D demonstrates that JQ1 does not alter BRD4 levels or ChIP-efficency. Western blot of relative BRD4 levels after 6 hours of JQ1 or DMSO treatment. ChIP-Western blot of the relative levels of immunoprecipitated BRD4 after 6 hours of JQ1 or DMSO treatment. FIG. **8**E demonstrates that super-enhancers show a greater loss of BRD4 occupancy when compared to regions with average or low amounts of BRD4. FIG. **8**F demonstrates that the IgH enhancer shows significantly greater loss of BRD4 than regions with lower BRD4 occupancy. Gene tracks of BRD4 at the IGH super enhancer and the average, CD28 enhancer after 6 hours of DMSO or JQ1 treatment.

FIGS. **9**A, **9**B and **9**C demonstrate that the loss of P-TEFb accompanies BRD4 inhibition. FIG. **9**A demonstrates that P-TEFb generally occupies enhancers bound by Mediator and BRD4 in MM1.S cells. FIG. **9**B demonstrates that the loss of BRD4 following JQ1 treatment is accompanied by loss of P-TEFb at enhancers. FIG. **9**C demonstrates that P-TEFb is disproportionally lost at super-enhancers.

FIGS. **10**A, **10**B and **10**C demonstrate that JQ1 causes disproportionate loss of transcription at super-enhancer genes. FIG. **10**A demonstrates that JQ1 leads to a global defect in transcription elongation. FIG. **10**B demonstrates that genes associated with super-enhancers show a dramatic defect in elongation. Gene tracks of RNA PolII occupancy at the MYC gene after 6 hour treatment with JQ1. FIG. **10**C demonstrates that genes associated with super enhancers show a larger increase in travelling ratio in response to JQ1 compared to genes associated with normal enhancers.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates in some aspects to super-enhancers and related compositions, methods, and agents that are useful for modulating expression of cell type-specific genes that are required for maintenance of cell identity (e.g., embryonic stem cell identity) or maintenance of a disease state (e.g., cancer). In some aspects, the present invention relates to methods of identifying super-enhancers, super-enhancer-associated genes, and disease-related genes in cells, tissues, organs and individuals, and kits comprising reagents for performing those methods.

During the course of work described herein, more than 200 genomic regions that contained tightly spaced clusters of enhancers spanning extraordinarily large domains were discovered. These "super-enhancers" are occupied by more transcriptional coactivator (e.g., Mediator) than the average or median enhancers, exhibit greater activity than average enhancers, and are sufficient to drive high expression of key, cell type-specific genes required to maintain cell identity or disease state.

Accordingly, in some aspects, the present invention relates to an isolated super-enhancer, or functional fragment and/or variant thereof, comprising a genomic region of DNA that contains at least two enhancers, wherein the genomic region is occupied when present within a cell by more super-enhancer component, e.g., transcriptional coactivator than the average single enhancer within the cell. As used herein, "enhancer" refers to a short region of DNA to which proteins (e.g., transcription factors) bind to enhance transcription of a gene. As used herein, "transcriptional coactivator" refers to a protein or complex of proteins that interacts with transcription factors to stimulate transcription of a gene. In some embodiments, the transcriptional coactivator is Mediator. In some embodiments, the transcriptional coactivator is Med1 (Gene ID: 5469). In some embodiments, the transcriptional coactivator is a Mediator component. As used herein, "Mediator component" comprises or consists of a polypeptide whose amino acid sequence is identical to the amino acid sequence of a naturally occurring Mediator complex polypeptide. The naturally occurring Mediator complex polypeptide can be, e.g., any of the approximately 30 polypeptides found in a Mediator complex that occurs in a cell or is purified from a cell (see, e.g., Conaway et al., 2005; Kornberg, 2005; Malik and Roeder, 2005). In some embodiments a naturally occurring Mediator component is any of Med1-Med31 or any naturally occurring Mediator polypeptide known in the art. For example, a naturally occurring Mediator complex polypeptide can be Med6, Med7, Med10, Med12, Med14, Med15, Med17, Med21, Med24, Med27, Med28 or Med30. In some embodiments a Mediator polypeptide is a subunit found in a Med11, Med17, Med20, Med22, Med8, Med18, Med19, Med6, Med30, Med21, Med4, Med7, Med31, Med10, Med1, Med27, Med26, Med14, Med15 complex. In some embodiments a Mediator polypeptide is a subunit found in a Med12/Med13/CDK8/cyclin complex. Mediator is described in further detail in PCT International Application No. WO 2011/100374, the teachings of which are incorporated herein by reference in their entirety. In some embodiments, Mediator occupation of an enhancer, e.g., a superenhancer, may be detected by detecting one or more Mediator components. It is to be understood that a Mediator inhibitor may inhibit one or more Mediator components or inhibit interaction(s) between them or inhibit interaction with a transcription factor.

In some embodiments a "naturally occurring polypeptide" is a polypeptide that naturally occurs in a eukaryote, e.g., a vertebrate, e.g., a mammal. In some embodiments the mammal is a human. In some embodiments the vertebrate is a non-human vertebrate, e.g., a non-human mammal, e.g., rodent, e.g., a mouse, rat, or rabbit. In some embodiments the vertebrate is a fish, e.g., a zebrafish. In some embodiments the eukaryote is a fungus, e.g., a yeast. In some embodiments the eukaryote is an invertebrate, e.g., an insect, e.g., a *Drosophila*,

or a nematode, e.g., *C. elegans*. Any eukaryotic species is encompassed in various embodiments of the invention. Similarly a cell or subject can be of any eukaryotic species in various embodiments of the invention. In some embodiments, the sequence of the naturally occurring polypeptide is the sequence most commonly found in the members of a particular species of interest. One of skill in the art can readily obtain sequences of naturally occurring polypeptides, e.g., from publicly available databases such as those available at the National Center for Biotechnology Information (NCBI) website (e.g., GenBank, OMIM, Gene).

In some embodiments, the transcriptional coactivator is a component of Mediator. In some embodiments, the Mediator component comprises a Med1 or a Med12 polypeptide. In some embodiments, the at least one Mediator component comprises Med6, Med7, Med10, Med12, Med14, Med15, Med17, Med21, Med24, Med27, Med28 and Med30 polypeptides.

In some embodiments, the genomic region of the super-enhancer is occupied when present within a cell by more chromatin regulator than the average single enhancer within the cell. As used herein, "chromatin regulator" refers to a protein or complex of proteins that is involved in regulating gene expression by interacting with transcription factors, transcriptional coactivators, and/or acetylated histone residues in a way that modulates expression of a super-enhancer-associated gene. In some instances, the chromatin regulator possesses histone acetyltransferase (HAT) activity. HATs are responsible for acetylating lysine residues on histone tails of nucleosomes, thereby relaxing the chromatin and increasing access to DNA. In some embodiments, the chromatin regulator is a BET bromodomain protein. In some embodiments, the BET bromodomain protein is BRD4 (Gene ID: 23476).

Generally, super-enhancers formed by the at least two enhancers in the genomic region of DNA are of greater length than the average single enhancer. In some embodiments, the length of the genomic region that forms the super-enhancer is at least an order of magnitude greater than the average single enhancer. In some embodiments, the genomic region spans between about 4 kilobases and about 40 kilobases in length. It should be appreciated, however, that super-enhancers may comprise genomic regions less than 4 kilobases or greater than 40 kilobases in length, as long as the genomic region contains clusters of enhancers that can be occupied when present within a cell by extremely high levels of a transcriptional coactivator (e.g., Mediator).

Table 1 (relating to nucleotide sequences of super-enhancers found within embryonic stem cells) and Table 2 (relating to nucleotide sequences of super-enhancers found within multiple myeloma cells); Table 3 (relating to nucleotide sequences of super-enhancers found in glioblastoma cells); and Table 4 (relating to nucleotide sequences of super-enhancers found in SCLC cells) disclose information that can be relied upon by one of skill in the art to obtain the specific nucleotide sequences for exemplary super-enhancers of the invention. For example, using the chromosomal number, and start and stop positions, as well as the sense orientation (e.g. +) of the sequence provided in Tables 1 and 2, one of skill in the art would be able to utilize a publicly available database (e.g., USCS Genome Browser, available at genome.ucsc.edu/) to obtain the nucleotide sequences of the specified super-enhancers. For the embryonic stem cell super-enhancer nucleotide sequences specified in Table 1, the mm9 genome build was used. This corresponds to NCBI build 37. For the multiple myeloma cell super-enhancer nucleotide sequences specified in Table 2, the hg 18 genome build was used. This corresponds to NCBI build 36. Tables 3 and 4 are also based on the hg 18 genome build. In some embodiments, the invention comprises a super-enhancer, or functional fragment and/or variant thereof, having a nucleotide sequence specified in Tables 1, 2, 3 or 4.

The at least two enhancers which form the super-enhancers, or functional fragment and/or variant thereof, are clustered together.

It should be appreciated that the each of the at least two enhancers can be the same type of enhancer or the at least two enhancers can be different types of enhancers. Each enhancer of the at least two enhancers comprises a binding site for a cognate transcription factor that interacts with the transcriptional coactivator to stimulate transcription of the gene associated with the super-enhancer. In some embodiments, the cognate transcription factor comprises an embryonic stem cell master transcription factor. Examples of suitable embryonic stem cell master transcription factors include, but are not limited to Oct4, Sox2, Nanog, Esrrb, Utf1, Klf4, mir-290-295 gene cluster, Tbx3, Sgk1, and combinations thereof. In some embodiments, the cognate transcription factor comprises an oncogenic transcription factor. Examples of suitable oncogenic transcription factors include, but are not limited to c-Myc, IRF4, p53, AP-1, Bcr-Ab1, c-Fos, c-Jun and combinations thereof. In some embodiments, the cognate transcription factor comprises a muscle cell transcription factor, for example, transcription factor MyoD. In some embodiments, the cognate transcription factor comprises a B cell transcription factor, for example Pu.1.

As noted above, the genomic region of the super-enhancers are occupied when present within a cell by more transcriptional coactivator (e.g., Mediator) and/or more chromatin regulator (e.g., BRD4) than the average single enhancer within the cell. In some embodiments, the genomic region of a super-enhancers is occupied when present within the cell by an order of magnitude more transcriptional coactivator or chromatin regulator than the average single enhancer in the cell. As used herein, "order of magnitude" refers to the relative fold difference in a feature or classification of one object as compared to a feature or classification of another object (e.g., a level or an amount of transcriptional coactivator occupying a super-enhancer associated with a gene as compared to the level or the amount of transcriptional coactivator occupying the average or median enhancer associated with the gene). In some embodiments, the order of magnitude is at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more. In some embodiments, the order of magnitude is at least 2-fold (i.e., there is a 2-fold greater amount of transcriptional coactivator occupying the super-enhancer associated with a gene than the amount of transcriptional coactivator occupying the average enhancer in the gene). In some embodiments, the order of magnitude is at least 10-fold. In some embodiments, the order of magnitude is at least 15-fold. In some embodiments, the order of magnitude is at least 16-fold.

Work described herein suggests that super-enhancers are a common feature of mammalian cells. Accordingly, the present invention contemplates that super-enhancers can be isolated from any mammalian cell type. Such isolation can be achieved by routine methods well known to those skilled in the art.

In some embodiments, super-enhancers of the present invention can be used drive high levels of expression of cell type specific genes. A cell type specific gene is typically expressed selectively in one or a small number of cells types relative to expression in many or most other cell types. One of skill in the art will be aware of numerous genes that are considered cell type specific. A cell type specific gene need

not be expressed only in a single cell type but may be expressed in one or several, e.g., up to about 5, or about 10 different cell types out of the approximately 200 commonly recognized (e.g., in standard histology textbooks) and/or most abundant cell types in an adult vertebrate, e.g., mammal, e.g., human. In some embodiments, a cell type specific gene is one whose expression level can be used to distinguish a cell of one of the following types from cells of the other cell types: adipocyte (e.g., white fat cell or brown fat cell), cardiac myocyte, chondrocyte, endothelial cell, exocrine gland cell, fibroblast, glial cell, hepatocyte, keratinocyte, macrophage, monocyte, melanocyte, neuron, neutrophil, osteoblast, osteoclast, pancreatic islet cell (e.g., a beta cell), skeletal myocyte, smooth muscle cell, B cell, plasma cell, T cell (e.g., regulatory, cytotoxic, helper), or dendritic cell. In some embodiments a cell type specific gene is lineage specific, e.g., it is specific to a particular lineage (e.g., hematopoietic, neural, muscle, etc.) In some embodiments, a cell-type specific gene is a gene that is more highly expressed in a given cell type than in most (e.g., at least 80%, at least 90%) or all other cell types. Thus specificity may relate to level of expression, e.g., a gene that is widely expressed at low levels but is highly expressed in certain cell types could be considered cell type specific to those cell types in which it is highly expressed. It will be understood that expression can be normalized based on total mRNA expression (optionally including miRNA transcripts, long non-coding RNA transcripts, and/or other RNA transcripts) and/or based on expression of a housekeeping gene in a cell. In some embodiments, a gene is considered cell type specific for a particular cell type if it is expressed at levels at least 2, 5, or at least 10-fold greater in that cell than it is, on average, in at least 25%, at least 50%, at least 75%, at least 90% or more of the cell types of an adult of that species, or in a representative set of cell types. One of skill in the art will be aware of databases containing expression data for various cell types, which may be used to select cell type specific genes. In some embodiments a cell type specific gene is a transcription factor.

In some aspects, the present invention relates to a composition comprising a super-enhancer of the present invention or a functional variant thereof. Such compositions may be useful for stimulating the expression of a gene or genes in a specific cell type, for example, to stimulate the expression of embryonic stem cell master transcription factors to maintain the cell in an embryonic stem cell-like state. In some instances, such compositions may be useful for stimulating the expression of a gene or genes in a specific cell type to change the identity of a specific cell-type, for example, by introducing a super-enhancer associated with a differentiated state to change the identity of an embryonic stem cell to a more differentiated state. In some embodiments, the super-enhancer can be used to stimulate expression of a target gene that is to be transfected into a cell for in vitro expression of that target gene. In some embodiments, the super-enhancer can be used to simulate a disease like state. By way of example, and not of limitation, an super-enhancer can be constructed using enhancers of an oncogene and transfection of the oncogene with the artificial enhancer can be useful to simulate the disease associated with the oncogene. Another exemplary use of a super-enhancer of the present invention is to identify genes that are prone to lead to disease upon aberrant expression. Such super-enhancers may be used in cells, tissues, organs, and whole organisms to artificially increase the expression of certain genes and examine the biological effects that the increased expression of the gene has on the cell, the tissue, organ, or animal.

It should be appreciated that any enhancer associated with the target gene can be cloned and used to form the super-enhancers. In some embodiments, the super-enhancer is engineered to mimic a super-enhancer identified in vivo, such as a super-enhancer that is responsible for maintaining embryonic stem cell identity, i.e., a super-enhancer comprising a plurality of Oct4, Sox2, and Nanog binding motifs oligomerized to form a concatemer.

In some aspects, the present invention relates to a nucleic acid construct comprising a super-enhancer, or functional fragment thereof, of the present invention. Methods of forming nucleic acid constructs are known to those skilled in the art. It should be understood that the nucleic acid constructs of the present invention are artificial or engineered constructs not to be confused with native genomic sequences. Such nucleic acid constructs can be used, for example, to increase the expression of a gene or genes associated with or regulated by the super-enhancer in the nucleic acid construct. In some instances, a nucleic acid construct comprising the super-enhancer can be introduced into a target cell and the super-enhancer can interact with endogenous cellular components to drive expression of an endogenous gene within the cell. In some embodiments, the nucleic acid construct includes a nucleotide sequence encoding a target gene operatively linked to the super-enhancer. In such instances, the nucleic acid can be transfected into a cell and interact with endogenous cellular components to drive expression of the exogenous target gene associated with the super-enhancer. In other embodiments, the nucleic acid construct can include a nucleic acid sequence encoding a transcriptional coactivator or chromatin regulator that can be expressed within the cell to produce transcriptional coactivator or chromatin regulator that can occupy the genomic region of the super-enhancer and increase expression of the gene associated with the super-enhancer in the cell. In some embodiments, the nucleic acid can include a reporter.

In some embodiments a reporter comprises a nucleic acid sequence that encodes a detectable marker, e.g., a fluorescent protein such as green fluorescent protein (GFP), blue, sapphire, yellow, red, orange, and cyan fluorescent proteins and fluorescent variants such as enhanced GFP (eGFP), mFruits such as mCherry, mTomato, mStrawberry; R-Phycoerythrin, etc. Enzymes useful as reporters include, e.g., enzymes that act on a substrate to produce a colored, fluorescent, or luminescent substance. Examples include luciferases, beta-galactosidase, horseradish peroxidase, and alkaline phosphatase. In some embodiments, alteration (e.g., reduction) in the level of a reporter may be used to identify a compound that modulates (e.g., inhibits) activity of a super-enhancer.

In some aspects, the present invention relates to a kit for increasing the expression of a gene, the kit including one or more or all of: (a) a population of cells; (b) reagents suitable for culturing said population of cells; (c) a nucleic acid construct comprising a super-enhancer enhancer or functional fragment and/or variant thereof, and a gene associated with the super-enhancer enhancer or functional fragment and/or variant thereof, that is capable of being expressed within said population of cells; and optionally (d) transcriptional coactivator or chromatin regulator e.g., excess levels of transcriptional coactivator or chromatin regulator that, e.g., can be introduced into said population of cells such that an order of magnitude more transcriptional coactivator or chromatin regulator occupies enhancers clustered within the super-enhancer and increases the expression of the gene within the cells.

In some aspects, the present invention relates to a cell, or cell-free system, into which a super-enhancer is introduced,

for example by transfection of a nucleic acid construct comprising the super-enhancer, wherein upon introduction of super-enhancer into the cell, or cell-free system, endogenous transcriptional coactivators and chromatin regulators within the cell co-occupy the enhancer clusters of the super-enhancer and the active transcription start sites of the target gene to stimulate expression of the target gene within the cell. It should be appreciated that the super enhancer, or functional fragment and/or variant thereof, may be associated with and regulate an endogenous gene within the transfected cell. In such instances, the gene regulated by the super-enhancer, or functional fragment and/or variant thereof, need not be introduced into the cell with the super-enhancer, for example a nucleic acid construct need not include a target gene for expression within the transfected cell. In other instances, such as when an exogenous gene is desired to be introduced within the transfected cell, or cell-free system, the exogenous gene can be introduced into the cell with the super-enhancer, or functional fragment and/or variant thereof, or functional fragment and/or variant thereof. It should be appreciated that the exogenous gene and the super-enhancer or functional fragment and/or variant thereof, can be introduced into the cell by any method and in any form (e.g., protein or nucleic acid). The exogenous gene and the super-enhancer, or functional fragment and/or variant thereof, can be introduced into the cell, or cell-free system, together or separately, for example a nucleic acid construct comprising the super enhancer, or functional fragment and/or variant thereof, may be further engineered to include an exogenous gene operatively linked to the super-enhancer, or functional fragment and/or variant thereof, and which is also capable of being expressed within the transfected cell, or cell-free system. In some embodiments, exogenous transcriptional coactivators and/or chromatin regulators can be introduced into the transfected cell, or cell-free system to ensure that the enhancer clusters of the super-enhancer and the active transcription start sites are co-occupied within the transfected cell, or cell-free system by more transcriptional coactivator and/or the chromatin regulator and thereby drive high levels of expression of either an exogenous or endogenous gene in the transfected cell, or cell-free system.

The super-enhancer and/or a nucleic acid construct comprising the super-enhancer, or functional fragment and/or variant thereof, can be transfected into any cell suitable for expressing the gene associated with the super-enhancer. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is an embryonic stem cell or embryonic stem cell-like cell. In some embodiments, the cell is a muscle cell. In some embodiments, the muscle cell is a myotube. In some embodiments, the cell is a B cell. In some embodiments, the B cell is a Pro-B cell.

In some aspects, the present invention relates to a functional variant of a super-enhancer. A variant may be shorter or longer than the original super-enhancer. The term "variant" encompasses "fragments" or "functional fragments" of super-enhancers, or functional sequence variants, of super-enhancers. A "fragment" is a continuous portion of a polypeptide or polynucleotide that is shorter than the original polypeptide or polynucleotide. In some embodiments a variant comprises or consists of a fragment. In some embodiments a fragment or variant is at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more as long as the original polypeptide or polynucleotide. A fragment may be an N-terminal, C-terminal, or internal fragment. A functional fragment of a super-enhancer can have one or more of the following properties:

a) when associated with a gene, e.g., a gene with which it is normally associated, it provides at least 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the level of expression as is seen with the intact super-enhancer;

b) when associated with a gene, e.g., a gene with which it is normally associated, it provides at least 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the level of binding of an super-enhancer component;

c) when associated with a gene, e.g., a gene with which it is normally associated, it provides at least 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the level of binding of a mediator protein, e.g., Med1;

d) it comprises at least 10, 20, 30, 40, 5, 60, 70, 80 or 90% of the enhancers of the super-enhancer of which it is a functional fragment; or

e) it is at least 10, 20, 30, 40, 5, 60, 70, 80 or 90% as long as the super-enhancer of which it is a functional fragment.

The term variant also encompasses "sequence variants," e.g., "functional sequence variants," of a super enhancer or fragment or functional fragment of a super-enhancer. A functional sequence variant of a super-enhancer can have one or more of the following properties:

a) it comprises sufficient nucleotide sequence homology or identity with a reference super-enhancer, e.g., the super-enhancer from which it is derived, that when associated with a gene, e.g., a gene with which the reference super-enhancer is normally associated, it provides at least 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the level of expression as is seen with the reference super-enhancer;

b) when associated with a gene, e.g., a gene with which the reference super-enhancer, e.g., the super-enhancer from which it is derived, is normally associated, it provides at least 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the level of binding of an super-enhancer component as is seen with the reference super-enhancer;

c) when associated with a gene, e.g., a gene with which the reference super-enhancer, e.g., the super-enhancer from which it is derived, is normally associated, it provides at least 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the level of binding of a mediator protein, e.g., Med1 as is seen with the reference super-enhancer;

d) it comprises at least 10, 20, 30, 40, 5, 60, 70, 80 or 90% of the number of functional enhancers as is seen with the reference super-enhancer, e.g., the super-enhancer from which it is derived;

e) it comprises at least 40, 50, 60, 70, 80, 90, 95, 97, or 99% sequence homology or identity with a reference super-enhancer, e.g., the super-enhancer from which it is derived;

f) it comprises at least 40, 50, 60, 70, 80, 90, 95, 97, or 99% sequence homology or identity, across its encompassed enhancer elements, with a reference super-enhancer, e.g., the super-enhancer from which it is derived; or

f) it comprises a first level or sequence or homology or identity across its encompassed enhancer elements and/or associated protein encoding element, and a second level of homology across untranslated and/or untranscribed regions between its encompassed enhancers, with a reference super-enhancer, e.g., the super-enhancer from which it is derived, wherein the first and second levels are independently selected from at least 40, 50, 60, 70, 80, 90, 95, 97, or 99% sequence homology or identity, and, e.g., the first level is higher than the second level, e.g., the first level is at least 80, 90, 95, 97, or 99% and the second level is at least 40, 50, or 60%.

In some embodiments a variant polypeptide comprises or consists of at least one domain of an original polypeptide. In some embodiments a variant polynucleotide hybridizes to an original polynucleotide under stringent conditions, e.g., high

stringency conditions, for sequences of the length of the original polypeptide. In some embodiments a variant polypeptide or polynucleotide comprises or consists of a polypeptide or polynucleotide that is at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more identical in sequence to the original polypeptide or polynucleotide over at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the original polypeptide or polynucleotide. In some embodiments a variant polypeptide comprises or consists of a polypeptide that is at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more identical in sequence to the original polypeptide over at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the original polypeptide, with the proviso that, for purposes of computing percent identity, a conservative amino acid substitution is considered identical to the amino acid it replaces. In some embodiments a variant polypeptide comprises or consists of a polypeptide that is at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more identical to the original polypeptide over at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the original polypeptide, with the proviso that any one or more amino acid substitutions (up to the total number of such substitutions) may be restricted to conservative substitutions. In some embodiments a percent identity is measured over at least 100; 200; 300; 400; 500; 600; 700; 800; 900; 1,000; 1,200; 1,500; 2,000; 2,500; 3,000; 3,500; 4,000; 4,500; or 5,000 amino acids. In some embodiments the sequence of a variant polypeptide comprises or consists of a sequence that has N amino acid differences with respect to an original sequence, wherein N is any integer between 1 and 10 or between 1 and 20 or any integer up to 1%, 2%, 5%, or 10% of the number of amino acids in the original polypeptide, where an "amino acid difference" refers to a substitution, insertion, or deletion of an amino acid. In some embodiments a difference is a conservative substitution. Conservative substitutions may be made, e.g., on the basis of similarity in side chain size, polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. In some embodiments, conservative substitutions may be made according to Table A, wherein amino acids in the same block in the second column and in the same line in the third column may be substituted for one another other in a conservative substitution. Certain conservative substitutions are substituting an amino acid in one row of the third column corresponding to a block in the second column with an amino acid from another row of the third column within the same block in the second column.

TABLE A

Aliphatic	Non-polar	G A P I L V
	Polar - uncharged	C S T M N Q
	Polar - charged	D E K R
Aromatic		H F W Y

In some aspects, the present invention relates to a method of increasing the level of expression of a target gene in a cell, the method including transfecting a cell under conditions suitable for expression of the target gene with a nucleic acid expression construct comprising a nucleic acid sequence encoding the target gene operatively linked to a super-enhancer, wherein upon transfection of the cell endogenous transcriptional coactivators and chromatin regulators within the cell co-occupy enhancers clustered within the super-enhancer and active transcription start sites of the target gene to increase the level of expression of the target gene within the cell. Those skilled in the art will appreciate that the step of transfecting can be achieved in a variety of ways according to well-known and routine methods, for example, by using a transfection reagent, such as a plasmid or a lipid based transfection reagent. In some instances, it may be desirable to introduce into the cell exogenous transcriptional coactivators and chromatin regulators to ensure that enhancers clustered within the super-enhancer and the activate transcription start sites of the target gene are co-occupied by an order of magnitude more of the transcriptional coactivators and chromatin regulators than the average enhancer of the target gene. The exogenous transcriptional coactivators and chromatin regulators can be introduced into the target cell in the form of nucleic acids that can be transfected into the cell for expression within the cell or in the form of proteins, for example, by microinjecting the proteins into the cell. Other ways of introducing nucleic acids and proteins into a cell are apparent to those skilled in the art. Upon transfection of the cell with the nucleic acid construct containing the super enhancer, or functional fragment and/or variant thereof, it is expected that the level of expression of the target gene will increase significantly, for example, the level of expression of the target gene is increased 2-fold, 3-fold, 4-fold, 5-fold, 6-fold or more within the cell.

In some aspects, the present invention relates to a kit for increasing the expression of a target gene in a cell, comprising one or more or all of: (a) a super-enhancer operatively linked to a target gene; (b) a population of cells suitable for expression of said target gene; and (c) a reagent for introducing the super-enhancer and the target gene into said population of cells. In some embodiments, the reagent comprises a transfection reagent, e.g., a plasmid.

In some aspects, the present invention relates to a kit for increasing the expression of a target gene in a cell, comprising one or more or all of: (a) a nucleic acid construct comprising an artificial super-enhancer operatively linked to the target gene; (b) a population of cells suitable for expression of said target gene; and (c) a reagent for transfecting said population of cells with said nucleic acid construct.

In some aspects, the invention relates to a nucleic acid vector comprising a super-enhancer, or functional fragment and/or variant thereof, and a site, e.g., a restriction enzyme site, disposed such that insertion of a structural gene at the site places the structural gene under the control of the super-enhancer, or functional fragment and/or variant thereof. In embodiments the vector further comprise one or more of a first selectable marker, a second selectable marker, and an origin of replication.

In some aspects, the invention relates to a nucleic acid vector comprising a super-enhancer or functional fragment and/or variant thereof, functionally linked to a heterologous reporter gene, e.g., a fluorescent protein e.g., GFP, or an enzyme, e.g., horse radish peroxidase. In embodiments the vector further comprise one or more of a first selectable marker, a second selectable marker, and an origin of replication. In some aspects, the invention relates to a kit comprising one or both of:

a first nucleic acid comprising a reference super-enhancer or functional fragment and/or variant thereof, optionally, coupled to a reporter gene; and a second nuclide acid comprising a site for insertion of an SE, or functional fragment and/or variant thereof, optionally, coupled to a reporter gene.

In some aspects, the invention relates to a kit comprising one or both of:

a nucleic acid vector comprising a super-enhancer, or functional fragment and/or variant thereof, functional linked to a heterologous reporter gene, e.g., a fluorescent protein e.g., GFP, or an enzyme, e.g., horse radish peroxidase. In embodiments the vector further comprise one or more of a first selectable marker, a second selectable marker, and an origin of replication; and

a nucleic acid vector comprising an site, e.g., an restriction enzyme site, and a reporter gene, e.g., a fluorescent protein e.g., GFP, or an enzyme, e.g., horse radish peroxidase, wherein said site is disposed such that insertion into the site of a super-enhancer, or functional fragment and/or variant thereof, will place the reporter gene under the control of the super-enhancer, or functional fragment and/or variant thereof. In embodiments the vector further comprise one or more of a first selectable marker, a second selectable marker, and an origin of replication.

In some aspects, the present invention relates to a method of identifying a super-enhancer in a cell, or cell-free system, comprising: (a) identifying a genomic region of a target gene within said cell, or cell-free system characterized by clusters of enhancers for binding cognate transcription factors capable of interacting with Mediator to stimulate transcription of the target gene within said cell, or cell-free system; (b) measuring in the identified genomic region a level of Mediator occupying said enhancers; and (c) identifying the genomic region as a super-enhancer if the level of Mediator occupying the clusters of enhancers is an order of magnitude more than the level of Mediator occupying the average enhancer of the target gene.

In other aspects, the present invention relates to a method of identifying a super-enhancer associated with a target gene, comprising: (a) analyzing the target gene for a genomic region comprising clusters of enhancers occupied by an order of magnitude more Mediator than an average enhancer of the target gene; and (b) identifying the genomic region as a super-enhancer associated with the target gene if said clusters of enhancers are occupied by the order of magnitude more Mediator than the average enhancer of the target gene In some embodiments, the order of magnitude is at least 2-fold, 10-fold, at least 15-fold, at least 16-fold, or more.

In some aspects, the present invention relates to a method of identifying a gene, e.g., a key gene or genes, that control a cell state or identity, e.g., contributes to unwanted proliferation, e.g., which contributes to a cancerous cell state, comprising:

(a) identifying a super-enhancer, e.g., within an animal, cell, or cell-free system; and

(b) identifying a gene or genes associated with the super-enhancer, e.g., a gene or genes within a range of proximity to the super-enhancer.

In an embodiment gene or genes that are within a certain proximity to the super-enhancer are identified as a putative key gene or genes that control the cell state or identity.

In an embodiment the method is performed in a cell-free system.

In an embodiment the method is performed in a cell preparation, e.g., a cultured cell preparation.

In an embodiment the method is performed in an animal model.

In an embodiment the method is first performed in a cell-free system, and repeated in a cell preparation, e.g., a cultured cell preparation.

In an embodiment the method is first performed in a cell-free system, or a cell preparation, e.g., a cultured cell preparation, and repeated in an animal.

In an embodiment the cell is a disease state cell, e.g., a cancer cell.

In an embodiment the cell-free system is derived from a disease state cell, e.g., a cancer cell.

In an embodiment, the identified gene is tested as a target for therapy, e.g., by administering an antagonist or inhibitor, e.g., an siRNA, of the product of the gene, to a cell or animal.

The range of proximity to the super-enhancer can extend as far as about 10 megabases (mb) upstream to one end of the super-enhancer to as far as about 10 mb downstream to the other end of the super-enhancer, and any range therebetween, for example 9 mb upstream to 9 mb downstream, 8 mb upstream to 8 mb downstream, 7 mb upstream to 7 mb downstream, 6 mb upstream to 6 mb downstream, 5 mb upstream to 5 mb downstream, 4 mb upstream to 4 mb downstream, 3 mb upstream to 3 mb downstream, 2 mb upstream to 2 mb downstream to 1 mb upstream to 1 mb downstream, or between 0.5 mb upstream and 0.5 mb downstream, 0.1 mb upstream to 0.1 mb downstream. It should be appreciated that the key genes could also, in some instances, overlap with the super-enhancer region. It is also to be understood that the range of proximity will increase or decrease depending on the length or size of the super-enhancer region, for example, if the super enhancer is 10 kb in length, then the upstream range of proximity extends as far as about 10 mb upstream to the most upstream portion of the 10 kb super-enhancer. Similarly, the downstream range of proximity would extend as far as about 10 mb downstream from the most downstream portion of the 10 kb super-enhancer. In some embodiments, the method of identifying key genes that control the cell state or identity involves measuring the expression of those genes in the cell in the presence and absence of an agent that disrupts the function of the super-enhancer identified, as well as assaying the cell for changes in its cell state or identity (e.g., from a more differentiated state to a less differentiated state, or from a healthy state to a diseased state). If the expression of a gene within the range of proximity is statistically significant when the super-enhancer is properly functioning but its expression decreases or becomes unremarkable in the presence of the agent that disrupts the super-enhancer function, then it is likely that the particular gene is a key gene that controls the cell state or identity, especially if its absence of expression is correlated to a change in the state or identity of the cell.

The aforementioned methods of identifying super-enhancers within a cell and identifying a super-enhancer associated with a target gene can be achieved by a variety of different methods, as would be understood by a person skilled in the art. In some embodiments, the super-enhancer is identified by performing chromatin immunoprecipitation high-throughput sequencing (ChIP-Seq). Example 1 below describes an example of a protocol that can be used to carry out such methods in normal cells, such as embryonic stem cells, for example. Example 2 below describes an example of a protocol that can be used to carry out such methods in tumor cells, such as MM.1S cells, for example.

In certain aspects, the present invention relates to a method of identifying a disease related super-enhancer in a cell, tissue, or organ of an individual suspected of having said disease, comprising: (a) identifying a super-enhancer in said cell, tissue, or organ; (b) identifying a gene associated with said super-enhancer; and (c) and correlating said super-enhancer to said disease.

In certain aspects, the present invention relates to a method of characterizing a subject, e.g, a subject having or suspected of having a disorder, e.g., a proliferative disorder, e.g., cancer, comprising:

acquiring a subject tissue sample;

determining if a super-enhancer is associated with a gene, e.g., a preselected gene,

thereby characterizing said subject.

In an embodiment the method comprises determining the genes in the sample that are associated with a super-enhancer.

In an embodiment, the patient is selected, classified, diagnosed, treated, or prognosed, responsive to the pattern of genes, e.g., a preselected pattern, associated with a super-enhancers, e.g., where a plurality of genes, e.g., a plurality of preselected genes, are associated with super-enhancers.

In an embodiment, the determination comprises: crosslinking chromatin from the sample, and selecting, e.g., by immunoprecipitation, a target protein, e.g., an super-enhancer component.

In an embodiment the target protein is a Mediator protein.

In an embodiment the gene or preselected gene is an oncogene, a kinase, a gene that controls cell proliferation, e.g., a myc gene.

In an embodiment the gene or preselected gene is other than an oncogene, a kinase, a gene that controls cell proliferation, e.g., a myc gene.

In an embodiment the method comprises classifying the subject as having a super-enhancer associated with a gene, e.g., a preselected gene.

In an embodiment the sample comprises cancer cells.

In an embodiment the method comprises characterizing a subject by:

acquiring a subject tissue sample;

determining a gene in the sample is associated with a super-enhancer, thereby characterizing said subject.

In an embodiment, responsive to said determination, the method comprises selecting and/or administering a therapy to said subject.

In an embodiment, responsive to said determination, the method comprises selecting, classifying, diagnosing, or prognosing said subject.

In an embodiment, responsive to said determination, the method comprises classifying the subject for treatment with an agent that antagonizes or inhibits the product of the gene or preselected gene.

In an embodiment, responsive to said determination, the method comprises administering to the subject an agent that antagonizes or inhibits the product of the gene or preselected gene.

A reaction mixture comprising a patient sample comprising chromatin from a cancer cell and a probe capable of determining if a preselected gene is associated with an super-enhancer.

In certain aspects, the present invention relates to a method of modifying a cell state or identity, comprising introducing into the cell a super-enhancer that is required to stabilize the cell state or identity. It is to be understood that the super-enhancers of the present invention are capable of modifying the cell state or identity of any cell in which it has been shown that the super-enhancer is required to stabilize the cell state or identity. In some embodiments, the cell state is an embryonic-stem cell like state. Upon introduction of the super-enhancer into the cell, the super-enhancer drives expression of genes that are required to maintain the cell state or identity associated with the super-enhancer.

In some aspects, cell state reflects the fact that cells of a particular type can exhibit variability with regard to one or more features and/or can exist in a variety of different conditions, while retaining the features of their particular cell type and not gaining features that would cause them to be classified as a different cell type. The different states or conditions in which a cell can exist may be characteristic of a particular cell type (e.g., they may involve properties or characteristics exhibited only by that cell type and/or involve functions performed only or primarily by that cell type) or may occur in multiple different cell types. Sometimes a cell state reflects the capability of a cell to respond to a particular stimulus or environmental condition (e.g., whether or not the cell will respond, or the type of response that will be elicited) or is a condition of the cell brought about by a stimulus or environmental condition. Cells in different cell states may be distinguished from one another in a variety of ways. For example, they may express, produce, or secrete one or more different genes, proteins, or other molecules ("markers"), exhibit differences in protein modifications such as phosphorylation, acetylation, etc., or may exhibit differences in appearance. Thus a cell state may be a condition of the cell in which the cell expresses, produces, or secretes one or more markers, exhibits particular protein modification(s), has a particular appearance, and/or will or will not exhibit one or more biological response(s) to a stimulus or environmental condition. Markers can be assessed using methods well known in the art, e.g., gene expression can be assessed at the mRNA level using Northern blots, cDNA or oligonucleotide microarrays, or sequencing (e.g., RNA-Seq), or at the level of protein expression using protein microarrays, Western blots, flow cytometry, immunohistochemistry, etc. Modifications can be assessesed, e.g., using antibodies that are specific for a particular modified form of a protein, e.g., phospho-specific antibodies, or mass spectrometry.

Another example of cell state is "activated" state as compared with "resting" or "non-activated" state. Many cell types in the body have the capacity to respond to a stimulus by modifying their state to an activated state. The particular alterations in state may differ depending on the cell type and/or the particular stimulus. A stimulus could be any biological, chemical, or physical agent to which a cell may be exposed. A stimulus could originate outside an organism (e.g., a pathogen such as virus, bacteria, or fungi (or a component or product thereof such as a protein, carbohydrate, or nucleic acid, cell wall constituent such as bacterial lipopolysaccharide, etc) or may be internally generated (e.g., a cytokine, chemokine, growth factor, or hormone produced by other cells in the body or by the cell itself). For example, stimuli can include interleukins, interferons, or TNF alpha. Immune system cells, for example, can become activated upon encountering foreign (or in some instances host cell) molecules. Cells of the adaptive immune system can become activated upon encountering a cognate antigen (e.g., containing an epitope specifically recognized by the cell's T cell or B cell receptor) and, optionally, appropriate co-stimulating signals. Activation can result in changes in gene expression, production and/or secretion of molecules (e.g., cytokines, inflammatory mediators), and a variety of other changes that, for example, aid in defense against pathogens but can, e.g., if excessive, prolonged, or directed against host cells or host cell molecules, contribute to diseases. Fibroblasts are another cell type that can become activated in response to a variety of stimuli (e.g., injury (e.g., trauma, surgery), exposure to certain compounds including a variety of pharmacological agents, radiation, etc.) leading them, for example, to secrete extracellular matrix components. In the case of response to injury, such ECM components can contribute to wound healing. However, fibroblast activation, e.g., if prolonged, inappropriate, or excessive, can lead to a range of fibrotic conditions affecting diverse tissues and organs (e.g., heart, kidney, liver, intestine, blood vessels, skin) and/or contribute to cancer. The presence of abnormally large amounts of ECM com-

ponents can result in decreased tissue and organ function, e.g., by increasing stiffness and/or disrupting normal structure and connectivity.

Another example of cell state reflects the condition of cell (e.g., a muscle cell or adipose cell) as either sensitive or resistant to insulin. Insulin resistant cells exhibit decreased respose to circulating insulin; for example insulin-resistant skeletal muscle cells exhibit markedly reduced insulin-stimulated glucose uptake and a variety of other metabolic abnormalities that distinguish these cells from cells with normal insulin sensitivity.

As used herein, a "cell state associated gene" is a gene the expression of which is associated with or characteristic of a cell state of interest (and is often not associated with or is significantly lower in many or most other cell states) and may at least in part be responsible for establishing and/or maintaining the cell state. For example, expression of the gene may be necessary or sufficient to cause the cell to enter or remain in a particular cell state.

In some aspects, modulating a super-enhancer function shifts a cell from an "abnormal" state towards a more "normal" state. In some embodiments, modulating a super-enhancer function shifts a cell from a "disease-associated" state towards a state that is not associated with disease. A "disease-associated state" is a state that is typically found in subjects suffering from a disease (and usually not found in subjects not suffering from the disease) and/or a state in which the cell is abnormal, unhealthy, or contributing to a disease.

In some embodiments, the methods and compounds herein are of use to reprogram a somatic cell, e.g., to a pluripotent state. In some embodiments the methods and compounds are of use to reprogram a somatic cell of a first cell type into a different cell type. In some embodiments, the methods and compounds herein are of use to differentiate a pluripotent cell to a desired cell type.

In an embodiment, the method of modifying a cell state or identity can be used to reprogram a cell to a less differentiated state, such method comprising the steps of:

(a) contacting a differentiated cell or population of cells with at least one reprogramming agent capable of reprogramming said cell to less differentiated state;

(b) maintaining said cell or population of cells under conditions appropriate for proliferation of said cell population and for activity of said at least one reprogramming agent for a period of time sufficient to begin reprogramming of said cell or population of cells; and (c) transfecting said cell or population of cells with a nucleic acid construct comprising a super-enhancer having a plurality of binding sites for cognate transcription factors Oct4, Sox2, and Nanog, wherein transfection of said cell drives high levels of expression of embryonic stem cell genes required to reprogram and maintain the cell in a less differentiated state. In an embodiment, the less differentiated state is an embryonic stem cell-like state. Reprogramming of cells and suitable reprogramming agents (e.g., Oct4, Sox2, Nanog, etc.) are described in further detail in U.S. Patent Application Publication No. 2011/0076678, U.S. Pat. No. 7,682,828, U.S. Pat. No. 8,071,369, U.S. Patent Application Publication No. 2012/0028821, U.S. Patent Application Ser. No. 61/098,327, the teachings of all of which are incorporated herein by reference in their entirety.

In certain aspects, the present invention relates to a kit for reprogramming a differentiated somatic cell population to an embryonic stem-cell like state, comprising: (a) a population of differentiated somatic cells; (b) at least one reprogramming agent capable of reprogramming said cell to an embryonic stem cell-like state; and (c) a nucleic acid construct comprising a super-enhancer containing clusters of enhancers having binding sites for cognate transcription factors Oct4, Sox2, and Nanog; and (d) a reagent for transfecting said population of cells with said nucleic acid construct.

In some embodiments, modulating a function (activity) of a super-enhancer is of use to treat, e.g., a metabolic, neurodegenerative, inflammatory, auto-immune, proliferative, infectious, cardiovascular, musculoskeletal, or other disease. It will be understood that diseases can involve multiple pathologic processes and mechanisms and/or affect multiple body systems. Discussion herein of a particular disease in the context of a particular pathologic process, mechanism, cell state, cell type, or affected organ, tissue, or system, should not be considered limiting. For example, a number of different tumors (e.g., hematologic neoplasms such as leukemias) arise from undifferentiated progenitor cells and/or are composed largely of undifferentiated or poorly differentiated cells that retain few if any distinctive features characteristic of differentiated cell types. These tumors, which are sometimes termed undifferentiated or anaplastic tumors, may be particularly aggressive and/or difficult to treat. In some embodiments of the invention, a method of the invention is used to modify such cells to a more differentiated state, which may be less highly proliferative and/or more amenable to a variety of therapies, e.g., chemotherapeutic agents. In another embodiment, an inventive method is used to treat insulin resistance which occurs, for example, in individuals suffering from type II diabetes and pre-diabetic individuals. It would be beneficial to modify the state of insulin-resistant cells towards a more insulin-sensitive state, e.g., for purposes of treating individuals who are developing or have developed insulin resistance. In another embodiment, an inventive method is used to treat obesity.

Many inflammatory and/or autoimmune conditions may occur at least in part as a result of excessive and/or inappropriate activation of immune system cells. Autoimmune diseases include, e.g., Graves disease, Hashimoto's thyroiditis, myasthenia gravis, rheumatoid arthritis, sarcoidosis, Sjögren's syndrome, scleroderma, ankylosing spondylitis, type I diabetes, vasculitis, and lupus erythematosus. Furthermore, immune-mediated rejection is a significant risk in organ and tissue transplantation. Inflammation plays a role in a large number of diseases and conditions. Inflammation can be acute (and may be recurrent) or chronic. In general, inflammation can affect almost any organ, tissue, or body system. For example, inflammation can affect the cardiovascular system (e.g., heart), musculoskeletal system, respiratory system (e.g., bronchi, lungs), renal system, (e.g., kidneys), eyes, nervous system, gastrointestinal system (e.g., colon), integumentary system (e.g., skin), musculoskeletal system (e.g., joints, muscles), resulting in a wide variety of conditions and diseases. Chronic inflammation is increasingly recognized as an important factor contributing to atherosclerosis and degenerative diseases of many types. Inflammation influences the microenvironment around tumours and contributes, e.g., to tumor cell proliferation, survival and migration. Furthermore, chronic inflammation can eventually lead to fibrosis.

Exemplary inflammatory diseases include, e.g., adult respiratory distress syndrome (ARDS), atherosclerosis (e.g., coronary artery disease, cerebrovascular disease), allergies, asthma, cancer, demyleinating diseases, dermatomyositis, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis), inflammatory myopathies, multiple sclerosis, glomerulonephritis, psoriasis, pancreatitis, rheumatoid arthritis, sepsis, vasculitis (including phlebitis and arteritis, e.g., polyarteritis nodosa, Wegener's granulomatosis, Buerger's disease, Takayasu's arteritis, etc.). In some embodiments, a method of the invention is used to modify immune cell state to

reduce activation of immune system cells involved in such conditions and/or render immune system cells tolerant to one or more antigens. In one embodiment, dendritic cell state is altered. Promoting immune system activation using a method of the invention (e.g., in individuals who have immunodeficiencies or have been treated with drugs that deplete or damage immune system cells), potentially for limited periods of time, may be of benefit in the treatment of infectious diseases.

In other embodiments, activated fibroblasts are modified to a less activated cell state to reduce or inhibit fibrotic conditions or treat cancer.

Post-surgical adhesions can be a complication of, e.g., abdominal, gynecologic, orthopedic, and cardiothoracic surgeries. Adhesions are associated with considerable morbidity and can be fatal. Development of adhesions involves inflammatory and fibrotic processes. In some embodiments, a method of the invention is used to modify state of immune system cells and/or fibroblasts to prevent or reduce adhesion formation or maintenance.

In other embodiments, modifying cells to a more or less differentiated state is of use to generate a population of cells in vivo that aid in repair or regeneration of a diseased or damaged organ or tissue, or to generate a population of cells ex vivo that is then administered to a subject to aid in repair or regeneration of a diseased or damaged organ or tissue.

In some embodiments, cell type and/or cell state becomes modified over the course of multiple cell cycle(s). In some embodiments, cell type and/or cell state is stably modified. In some embodiments, a modified type or state may persist for varying periods of time (e.g., days, weeks, months, or indefinitely) after the cell is no longer exposed to the agent(s) that caused the modification. In some embodiments, continued or at intermittent exposure to the agent(s) is required or helpful to maintain the modified state or type.

Cells may be in living animal, e.g., a mammal, or may be isolated cells. Isolated cells may be primary cells, such as those recently isolated from an animal (e.g., cells that have undergone none or only a few population doublings and/or passages following isolation), or may be a cell of a cell line that is capable of prolonged proliferation in culture (e.g., for longer than 3 months) or indefinite proliferation in culture (immortalized cells). In many embodiments, a cell is a somatic cell. Somatic cells may be obtained from an individual, e.g., a human, and cultured according to standard cell culture protocols known to those of ordinary skill in the art. Cells may be obtained from surgical specimens, tissue or cell biopsies, etc. Cells may be obtained from any organ or tissue of interest. In some embodiments, cells are obtained from skin, lung, cartilage, breast, blood, blood vessel (e.g., artery or vein), fat, pancreas, liver, muscle, gastrointestinal tract, heart, bladder, kidney, urethra, prostate gland. Cells may be maintained in cell culture following their isolation. In certain embodiments, the cells are passaged or allowed to double once or more following their isolation from the individual (e.g., between 2-5, 5-10, 10-20, 20-50, 50-100 times, or more) prior to their use in a method of the invention. They may be frozen and subsequently thawed prior to use. In some embodiments, the cells will have been passaged or permitted to double no more than 1, 2, 5, 10, 20, or 50 times following their isolation from the individual prior to their use in a method of the invention. Cells may be genetically modified or not genetically modified in various embodiments of the invention. Cells may be obtained from normal or diseased tissue. In some embodiments, cells are obtained from a donor, and their state or type is modified ex vivo using a method of the invention. The modified cells are administered to a recipient, e.g., for cell therapy purposes. In some embodiments, the cells are obtained from the individual to whom they are subsequently administered.

A population of isolated cells in any embodiment of the invention may be composed mainly or essentially entirely of a particular cell type or of cells in a particular state. In some embodiments, an isolated population of cells consists of at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% cells of a particular type or state (i.e., the population is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% pure), e.g., as determined by expression of one or more markers or any other suitable method.

In certain aspects, the present invention relates to a method of selectively inhibiting expression of an aberrantly expressed gene comprising disrupting the function of a super-enhancer associated with the gene. In certain embodiments, the gene comprises an oncogene. During the course of work described herein, the present inventors have observed that disruption of super-enhancers by BRD4 inhibition led to a dramatic loss of expression of critical tumor genes, accompanied by a potent anti-proliferative effect. Given the fact that super-enhancers are common features of mammalian cells, and that super-enhancers have been shown to drive high levels of gene expression, it is reasonable to expect that super-enhancer disruption can be used to selectively inhibit expression of any gene (e.g., any gene that is overexpressed in a diseased cell, wherein the gene is associated with a super-enhancer) by disrupting the super-enhancer associated with the oncogene. In an embodiment, the oncogene is MYC. In an embodiment, the oncogene is IRF4.

It should be appreciated that the present invention contemplates the use of any technique or any agent that is capable of disrupting the function of the super-enhancer. Generally, disrupting the function of the super-enhancer involves contacting said super-enhancer region with an effective amount of an agent that interferes with occupancy of the super-enhancer region by a cognate transcription factor for the gene, a transcriptional coactivator, or a chromatin regulator. In some embodiments, disrupting the function of the super-enhancer can be achieved by contacting the super-enhancer region with a pause release agent. In certain embodiments, the agent interferes with a binding site on the super-enhancer for the cognate transcription factor, interferes with interaction between the cognate transcription factor and a transcriptional coactivator, inhibits the transcription coactivator, or interferes with or inhibits the chromatin regulator. In some embodiments, the agent is a bromodomain inhibitor. In some embodiments, the agent is a BRD4 inhibitor. In some embodiments, the agent is the compound JQ1. In some embodiments, the agent is iBET.

Any of a wide variety of agents (also termed "compounds") can be used to disrupt the function of the super-enhancer, such as BET bromodomain inhibitors, P-TEFb inhibitors or compounds that interfere with binding of the cognate transcription factors to the binding sites of the super-enhancer associated with the gene (e.g, if the gene is an oncogene, such as MYC, a c-Myc inhibitor can be used to disrupt the function of the super-enhancer). An inhibitor could be any compound that, when contacted with a cell, results in decreased functional activity of a molecule or complex, e.g., transcriptional coactivator (e.g., Mediator), a chromatin regulator (e.g., BRD4), an elongation factor (e.g., P-TEFb), or cognate transcription factor (e.g., a cognate oncogenic transcription factor), in the cell. An inhibitor could act directly, e.g., by physically interacting with a molecule or complex to be inhibited, or a component thereof, or indirectly such as by interacting

with a different molecule or complex required for activity of the molecule or complex to be inhibited, or by interfering with expression or localization.

Compounds of use in various embodiments of the invention can comprise, e.g., small molecules, peptides, polypeptides, nucleic acids, oligonucleotides, etc. Certain non-limiting examples are presented below.

A small molecule is often an organic compound having a molecular weight equal to or less than 2.0 kD, e.g., equal to or less than 1.5 kD, e.g., equal to or less than 1 kD, e.g., equal to or less than 500 daltons and usually multiple carbon-carbon bonds. Small molecules often comprise one or more functional groups that mediate structural interactions with proteins, e.g., hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, and in some embodiments at least two of the functional chemical groups. A small molecule may comprise cyclic carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more chemical functional groups and/or heteroatoms. In some embodiments a small molecule satisfies at least 3, 4, or all criteria of Lipinski's "Rule of Five". In some embodiments, a compound is cell-permeable, e.g., within the range of typical compounds that act intracellularly, e.g., within mammalian cells. In some embodiments, the IC50 of a compound, e.g., a small molecule, for a target to be inhibited is less than or equal to about 5 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 10 μM, 50 μM, or 100 μM,

Nucleic acids, e.g., oligonucleotides (which typically refers to short nucleic acids, e.g., 50 nucleotides in length or less), the invention contemplates use of oligonucleotides that are single-stranded, double-stranded (ds), blunt-ended, or double-stranded with overhangs, in various embodiments of the invention. The full spectrum of modifications (e.g., nucleoside and/or backbone modifications), non-standard nucleotides, delivery vehicles and systems, etc., known in the art as being useful in the context of siRNA or antisense-based molecules for research or therapeutic purposes is contemplated for use in various embodiments of the instant invention. In some embodiments a compound is an RNAi agent, antisense oligonucleotide, or aptamer. The term "RNAi agent" encompasses nucleic acids that can be used to achieve RNA silencing in mammalian cells. As used herein RNA silencing, also termed RNA interference (RNAi), encompasses processes in which sequence-specific silencing of gene expression is effected by an RNA-induced silencing complex (RISC) that has a short RNA strand incorporated therein, which strand directs or "guides" sequence-specific degradation or translational repression of mRNA to which it has complementarity. The complementarity between the short RNA and mRNA need not be perfect (100%) but need only be sufficient to result in inhibition of gene expression. For example, the degree of complementarity and/or the characteristics of the structure formed by hybridization of the mRNA and the short RNA strand can be such that the strand can (i) guide cleavage of the mRNA in the RNA-induced silencing complex (RISC) and/or (ii) cause translational repression of the mRNA by RISC. The short RNA is often incorporated into RISC as part of a short double-stranded RNA (dsRNA). RNAi may be achieved artificially in eukaryotic, e.g., mammalian, cells in a variety of ways. For example, RNAi may be achieved by introducing an appropriate short double-stranded nucleic acid into the cells or expressing in the cells a nucleic acid that is processed intracellularly to yield such short dsRNA. Exemplary RNAi agents are a short hairpin RNA (shRNA), a short interfering RNA (siRNA), micrRNA (miRNA) and a miRNA precursor. siRNAs typically comprise two separate nucleic acid strands that are hybridized to each other to form a duplex. They can be synthesized in vitro, e.g., using standard nucleic acid synthesis techniques. A nucleic acid may contain one or more non-standard nucleotides, modified nucleosides (e.g., having modified bases and/or sugars) or nucleotide analogs, and/or have a modified backbone. Any modification or analog recognized in the art as being useful for RNAi, aptamers, antisense molecules or other uses of oligonucleotides can be used. Some modifications result in increased stability, cell uptake, potency, etc. Exemplary compound can comprise morpholinos or locked nucleic acids. In some embodiments the nucleic acid differs from standard RNA or DNA by having partial or complete 2'-O-methylation or 2'-O-methoxyethyl modification of sugar, phosphorothioate backbone, and/or a cholesterol-moiety at the 3'-end. In certain embodiments the siRNA or shRNA comprises a duplex about 19 nucleotides in length, wherein one or both strands has a 3' overhang of 1-5 nucleotides in length (e.g., 2 nucleotides), which may be composed of deoxyribonucleo tides. shRNA comprise a single nucleic acid strand that contains two complementary portions separated by a predominantly non-self-complementary region. The complementary portions hybridize to form a duplex structure and the non-self-complementary region forms a loop connecting the 3' end of one strand of the duplex and the 5' end of the other strand. shRNAs can undergo intracellular processing to generate siRNAs. In certain embodiments the term "RNAi agent" also encompasses vectors, e.g., expression vectors, that comprise templates for transcription of an siRNA (e.g., as two separate strands that can hybridize), shRNA, or microRNA precursor, and can be used to introduce such template into mammalian cells and result in transient or stable expression thereof.

In some embodiments an RNAi agent, aptamer, antisense oligonucleotide, other nucleic acid, peptide, polypeptide, or small molecule is physically associated with a moiety that increases cell uptake, such as a cell-penetrating peptide, or a delivery agent. In some embodiments a delivery agent at least in part protects the compound from degradation, metabolism, or elimination from the body (e.g., increases the half-life). A variety of compositions and methods can be used to deliver agents to cells in vitro or in vivo. For example, compounds can be attached to a polyalkylene oxide, e.g., polyethylene glycol (PEG) or a derivative thereof, or incorporated into or attached to various types of molecules or particles such as liposomes, lipoplexes, or polymer-based particles, e.g., microparticles or nanoparticles composed at least in part of one or more biocompatible polymers or copolymers comprising poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesterar des, polyorthoesters, polyhydroxybutyric acid, and/or polyanhydrides.

In some embodiments, an agent comprises a polypeptide. A "polypeptide" refers to a polymer of amino acids linked by peptide bonds. A protein is a molecule comprising one or more polypeptides. A peptide is a relatively short polypeptide, typically between about 2 and 100 amino acids (aa) in length, e.g., between 4 and 60 aa; between 8 and 40 aa; between 10 and 30 aa. The terms "protein", "polypeptide", and "peptide" may be used interchangeably. In general, a polypeptide may contain only standard amino acids or may comprise one or more non-standard amino acids (which may be naturally occurring or non-naturally occurring amino acids) and/or amino acid analogs in various embodiments. A "standard amino acid" is any of the 20 L-amino acids that are commonly utilized in the synthesis of proteins by mammals and are encoded by the genetic code. A "non-standard amino acid" is an amino acid that is not commonly utilized in the synthesis of proteins by mammals. Non-standard amino acids

include naturally occurring amino acids (other than the 20 standard amino acids) and non-naturally occurring amino acids. In some embodiments, a non-standard, naturally occurring amino acid is found in mammals. For example, ornithine, citrulline, and homocysteine are naturally occurring non-standard amino acids that have important roles in mammalian metabolism. Exemplary non-standard amino acids include, e.g., singly or multiply halogenated (e.g., fluorinated) amino acids, D-amino acids, homo-amino acids, N-alkyl amino acids (other than proline), dehydroamino acids, aromatic amino acids (other than histidine, phenylalanine, tyrosine and tryptophan), and α,α disubstituted amino acids. An amino acid, e.g., one or more of the amino acids in a polypeptide, may be modified, for example, by addition, e.g., covalent linkage, of a moiety such as an alkyl group, an alkanoyl group, a carbohydrate group, a phosphate group, a lipid, a polysaccharide, a halogen, a linker for conjugation, a protecting group, etc. Modifications may occur anywhere in a polypeptide, e.g., the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. A given polypeptide may contain many types of modifications. Polypeptides may be branched or they may be cyclic, with or without branching. Polypeptides may be conjugated with, encapsulated by, or embedded within a polymer or polymeric matrix, dendrimer, nanoparticle, microparticle, liposome, or the like. Modification may occur prior to or after an amino acid is incorporated into a polypeptide in various embodiments. Polypeptides may, for example, be purified from natural sources, produced in vitro or in vivo in suitable expression systems using recombinant DNA technology (e.g., by recombinant host cells or in transgenic animals or plants), synthesized through chemical means such as conventional solid phase peptide synthesis, and/or methods involving chemical ligation of synthesized peptides (see, e.g., Kent, S., J Pept Sci., 9(9):574-93, 2003 or U.S. Pub. No. 20040115774), or any combination of the foregoing.

One of ordinary skill in the art will understand that a protein may be composed of a single amino acid chain or multiple chains associated covalently or noncovalently. In some embodiments, the agent is a non-functional mutant of the cognate oncogenic transcription factor, the transcriptional coactivator, or the chromatin regulator that mimics interactions of the cognate oncogenic transcription factor, the transcriptional coactivator, or the chromatin regulator but lacks the ability to activate transcription of the oncogene. For example, a polypeptide can be a dominant negative version of Mediator, an elongation factor (e.g., P-TEFb subunit) or a dominant negative version of a cognate oncogenic transcription factor (e.g., a c-Myc or Max). A polypeptide that binds to and inhibits Mediator or P-TEFb or c-Myc could be identified, e.g., using phage display.

In some embodiments a compound comprises an antibody. The term "antibody" encompasses immunoglobulins and derivatives thereof containing an immunoglobulin domain capable of binding to an antigen. An antibody can originate from any mammalian or avian species, e.g., human, rodent (e.g., mouse, rabbit), goat, chicken, etc., or can be generated using, e.g., phage display. The antibody may be a member of any immunoglobulin class, e.g., IgG, IgM, IgA, IgD, IgE, or subclasses thereof such as IgG1, IgG2, etc. In various embodiments of the invention "antibody" refers to an antibody fragment such as an Fab', F(ab')2, scFv (single-chain variable) or other fragment that retains an antigen binding site, or a recombinantly produced scFv fragment, including recombinantly produced fragments. An antibody can be monovalent, bivalent or multivalent in various embodiments. The antibody may be a chimeric or "humanized" antibody, which can be generated using methods known in the art. An antibody may be polyclonal or monoclonal, though monoclonal antibodies may be preferred. Methods for producing antibodies that specifically bind to virtually any molecule of interest are known in the art. In some aspects the antibody is an intrabody, which may be expressed intracellularly. In some embodiments a compound comprises a single-chain antibody and a protein transduction domain (e.g., as a fusion polypeptide).

In some embodiments, a composition or method of the invention employs a transcriptional coactivator inhibitor, a chromatin regulator inhibitor, an elongation factor or pause release inhibitor, or a cognate transcription factor inhibitor that are small molecules.

In some embodiments, the agent is a BET bromodomain inhibitor. In some embodiments, the agent is a BRD4 inhibitor. In some embodiments, the agent is JQ1. In some embodiments, the agent is iBET. In some embodiments, the elongation factor or pause release inhibitor is a P-TEFb inhibitor. In some embodiments, the cognate oncogenic transcription factor inhibitor is a c-Myc inhibitor. In some embodiments, a composition or method employs a Mediator inhibitor, a BRD4 inhibitor, a P-TEFb inhibitor and a c-Myc inhibitor that each comprise a nucleic acid, e.g., RNAi agents. In some embodiments, a composition or method employs a Mediator inhibitor, a P-TEFb inhibitor that comprises a nucleic acid, e.g., RNAi agents, e.g., siRNAs. In some embodiments, the Mediator inhibitor may bind to a Mediator component, Mediator complex, or a Mediator associated protein, for example, an antibody directed against the Mediator component, Mediator complex, or the Mediator associated protein. Examples of suitable antibodies can be found in PCT International Application No. WO 2011/100374, the teachings of which are incorporated herein by reference in their entirety.

In some embodiments the material is isolated using an agent (e.g., an antibody) that binds to a Mediator component, Mediator complex, or that binds to a Mediator-associated protein.

In some embodiments, the agent is a nucleic acid that hybridizes to a binding site on the super-enhancer for the cognate transcription factor.

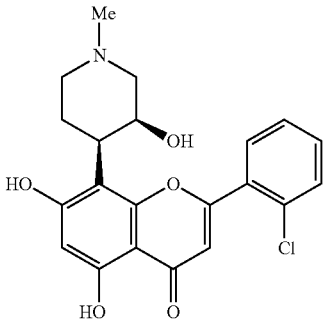
Compounds can be produced using any suitable method known in the art. The skilled artisan will select an appropriate method based, e.g., on the nature of the compound. The production method can be partially or completely synthetic in various embodiments. In some embodiments a compound (or starting material for synthesis) is purified from an organism or other natural source, e.g., a plant, microbe, fermentation broth, etc. A compound of use in the invention may be provided as part of a composition, which may contain, e.g., anion, salt, aqueous or non-aqueous diluent or carrier, buffer, preservative, etc. It is noted that although combined use of compounds is of particular interest, the use of compounds disclosed herein is not limited to their use in combination. In some embodiments of the invention, a compound may be used as a single agent.

In some embodiments, a P-TEFb inhibitor inhibits CDK9 kinase activity. The compound may inhibit one or more additional kinases, e.g., CDKs, in addition to CDK9. Often a kinase inhibitor acts by binding to an ATP binding pocket of a kinase. Thus in some embodiments a CDK9 inhibitor binds to the ATP binding pocket of CDK9. In some embodiments the P-TEFb inhibitor is selective for CDKs relative to many, most, or all other kinase families. In some embodiments the CDK inhibitor is selective for CDKs 1, 4, and 9 versus CDK2. In some embodiments the P-TEFb inhibitor is a CDK inhibitor that is selective for CDK9 versus CDK2. In some embodi-

ments the P-TEFb inhibitor is a CDK inhibitor that is selective for CDK9 versus CDK1 and CDK4. It will be appreciated that kinase inhibitory activity is tested against CKDs in complex with a preferred cyclin partner. For example, in some embodiments CDK2 activity can be tested using cyclin A. It will also be appreciated that a kinase assay can employ a relevant substrate, e.g., a physiologically relevant substrate or portion thereof comprising a phosphoryation site for the kinase.

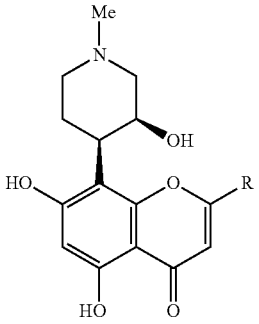
In some embodiments, the compound is an N-methylpiperidinyl, chlorophenyl flavone. In some embodiments, the compound is flavopiridol or a flavopiridol analog.

Flavopiridol (−)-2-(2-Chlorophenyl)-5,7-dihydroxy-8-[(3S,4R)-3-hydroxy-1-methyl-4-piperidinyl]-4H-1-benzopyran-4-one hydrochloride is a synthetic flavone that inhibits multiple CDKs, including CDK9. Its structure is shown below.



Flavopiridol has been shown to have antitumor activity against various tumor cells lines and to inhibit tumor growth in xenograft models. It has undergone clinical trials in a number of different cancer types including various solid tumors and leukemias. As described further in the examples, flavopiridol was shown to inhibit pause release. Without wishing to be bound by theory, this may help counteract the effects of Myc overexpression, and this may be the basis for the therapeutic effect of flavopiridol on some tumors.

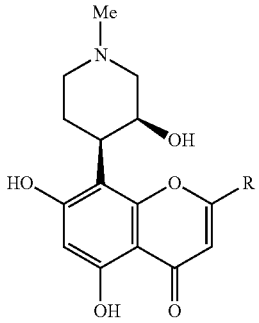
Flavopiridol analogs include compounds designed based on flavopiridol, e.g., by modifying one or more of the rings of the flavopiridol structure at one or more positions. In some embodiments, a flavopiridol analog is a 2-thio or 2-oxo flavopiridol analog. For example, PCT/US 1997/007610 describes compounds of formula I:



wherein X is oxygen or sulfur; R^1, R^2, R^3, R^4, R^5, R^6, m, and n are as defined in PCT/US1997/007610.

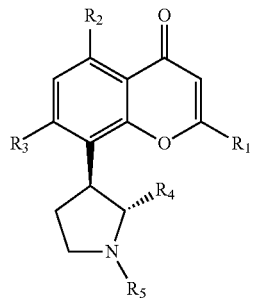
Additional flavopiridol analogs are disclosed in Murthi, K. K., et al., Bioorg Med Chem Lett. 10(10): 1037-41, 2000, which describes modifications of the 3-hydroxy-1-methylpiperidinyl (D ring) of flavopiridol.

In some embodiments, a flavopiridol analog has the following structure:

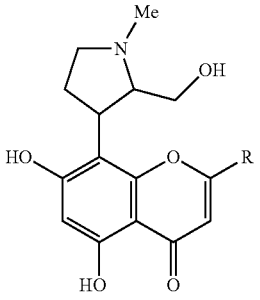


In some embodiments R is phenyl or substituted phenyl, e.g., halogenated phenyl. In some embodiments, R is selected from the group consisting of: 3-chlorophenyl, 4-chlorophenyl, 2-fluorophenyl, 4-fluorophenyl, 4-bromophenyl, 4-t-butylphenyl, 4-trifluoromethylphenyl, 4-hydroxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 4-chloro-3-pyridyl, 5-methylisoxazole, 3-vinylphenyl, 4-vinylphenyl, 2-chlorophenyl, 4-fluorophenyl, 2-bromophenyl, and 3-pyridyl. In some embodiments the compound displays increased selectivity for CDK9 than does flavopiridol. See, e.g., Ali, A., et al., Chembiochem, 10(12):2072-80, 2009, for additional information regarding these compound.

In some embodiments, a CDK9 inhibitor has the following structure:



wherein R1, R2, R3, R4, and R9 are as defined in PCT/IB 2006/052002 (WO/2007/148158). In some embodiments (i) RI comprises an aromatic group; (ii) R4 comprises an R—(OH) group, wherein R is a C_{1-6} aliphatic group; (iii) R9 comprises a C_{1-6} aliphatic group, e.g, a methyl group; or (iv) any combination of (i), (ii), and (iii). In some embodiments, the compound may have the following structure:



wherein R comprises an aromatic group.

Crystal structures of P-TEFb (CDK9/cyclin T1) alone and in a complex with flavopiridol are available (Baumli, S., et al., EMBO J. 27(13): 1907-18, 2008). Flavopiridol was shown to bind to the ATP binding pocket of CDK9. Structural information can be used in the design of additional P-TEFb inhibitors including, but not limited to, additional analogs of flavopiridol. Furthermore, virtual screening can be performed using structural information regarding diverse chemical compounds to identify candidate P-TEFb inhibitors. In some embodiments, a P-TEFb inhibitor is a compound that makes similar intermolecular contacts with CDK9 as does flavopiridol. Similar approaches can be used to design analogs of other CDK9 inhibitors.

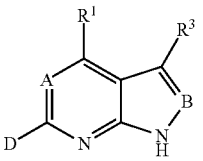
In some embodiments, a flavopiridol analog exhibiting reduced binding to human serum relative to flavopiridol is used.

In some embodiments, the P-TEFb inhibitor is a purine or purine analog, e.g., a biaryl purine analog. In some embodiments, the purine analog is a 2,6,9-substituted purine analog. In some embodiments, the compound is roscovitine, e.g., S-roscovitine or R-roscovitine. Unless otherwise indicated, where roscovitine is mentioned herein, the roscovitine can be R-roscovitine (also called Seliciclib or CYC202; 2-(R)-(1-Ethyl-2-hydroxyethy lamino)-6-benzylamino-9-isopropyl purine). Roscovitine is a CDK inhibitor that preferentially inhibit multiple enzyme targets including CDK1, CDK2, CDK7 and CDK9 and has been studied in clinical trials for treatment of a variety of proliferative diseases.

In some embodiments the compound is a roscovatine analog. Exemplary roscovitine analogs are oloumicine (2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine), olomoucine II (6-[(2-hydroxybenzyfiamino]-2-[[1-(hydroxymethyl)propyl]amino]-9-isopropylpurine) and LGR1406 (N-5-(2-aminocyclohexyl)-N-7-benzyl-3-isopropyl-1(2)H-pyrazolo[4,3-d]pyrimidine-5,7-di-amine). Roscovitine analogs generated by introduction of an aryl ring onto the 4-position of the C-6 benzyl amino group of roscovitine, and a series of C-6 biarylmethylamino derivatives prepared with modifications on the C-6 biaryl rings, N-9 and C-2 positions, are described in Trova, M P, et. al., Bioorg Med Chem Lett. 19(23):6608-12, 2009.

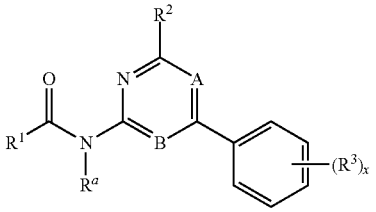
Many additional CDK inhibitors are known in the art that may inhibit CDK9, optionally with at least some selectivity relative to inhibition of one or more other CDKs. For example, PCT/US2009/049637 (WO/2010/003133) discloses compounds that are

reported to inhibit CDK9. In some aspects, the compounds have the following structure, where R1 and R3 are as defined therein.



(I)

PCT/EP2008/063715 (WO 2009047359) discloses additional compounds that are reported to inhibit CDK9. In some aspects, the compounds have the following structure, wherein R1, R2, Ra, and $(R3)_x$ are as defined therein.



In some embodiments, a P-TEFb inhibitor comprises an RNAi agent (e.g., an siRNA) or an antisense oligonucleotide that inhibits expression of a P-TEFb subunit (e.g., CDK9, cyclin T1, T2a, T2b, or K). In some embodiments a P-TEFb inhibitor comprises an antibody or aptamer that specifically binds to a P-TEFb subunit. Optionally the antibody or aptamer may bind to multiple CDKs or cyclins.

In some embodiments, a c-Myc inhibitor is a small molecule. In some embodiments, a c-Myc inhibitor inhibits formation of c-Myc/Max heterodimers. In some embodiments, a c-Myc inhibitor inhibits binding of c-Myc/Max to a target site in DNA. In some embodiments a c-Myc inhibitor is relatively specific for inhibiting transcription mediated by c-Myc relative to transcription mediated by many or most other basic helix-loop-helix/leucine zipper transcription factors.

Various compounds that inhibit c-Myc are described in Berg, T., Curr. Op. Chem. Biol., 12: 464-471, 2008, and references therein. The peptide mimetic IIA6B17 is described in Berg, T., et al., Proc Natl Acad Sci USA 99 (2002), pp. 3830-3835 and was shown to inhibit c-Myc-dependent transcription in a reporter gene assay (X. Lu, et al. Oncol Rep 19 (2008), pp. 825-830.). Testing a 285 member chemical library derived from planar, aromatic scaffolds in a c-Myc/Max dimerization assay led to identification of four structurally related Myc/Max dimerization inhibitors, which also inhibited DNA binding of c-Myc/Max (Y. Xu, et al. Bioorg Med Chem 14 (2006), pp. 2660-2673.) For example, the compound NY2267 strongly inhibited c-Myc-dependent oncogenic transformation of chicken embryo fibroblasts at 20 μM, showed selectivity over transformation mediated by v-Src or v-Jun, but did not discriminate between transcription mediated by c-Jun and c-Myc. Several compounds were selected from a chemical library on the basis of their ability to prevent association of the HLH-Zip domains of c-Myc and Max in a yeast two-hybrid assay (X. Yin, et al., Oncogene 22 (2003), pp. 6151-6159.). One, 10058-F4 (IC_{50}=49 μM on HL60 cells), served as starting point for the testing of derivatives with improved activities. One of the numerous derivatives resulting from structural variation of the substituents on the aromatic ring and the rhodanine moiety, the compound 28RH-NCN-1, inhibited DNA binding of c-Myc with activity comparable to that of the parent compound, and inhibited

growth of HL60 cells with improved potency (IC_{50}=29 μM) (Wang, H., et al., Mol Cancer Ther 6 (2007), pp. 2399-2408). See also PCT/US2007/004039 (WO/2007/098010).

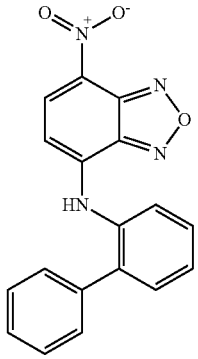
Screening chemical libraries for compounds that inhibited DNA binding of c-Myc, led to discovery of the pyrazolo[1,5-a]pyrimidine Mycro1 (Kiessling, A., et al., Chem Biol 13 (2006), pp. 745-751.). Mycro1 and the derivative Mycro2 were subsequently shown to inhibit c-Myc/Max dimerization, c-Myc-dependent proliferation, gene transcription, and oncogenic transformation. While Mycro1 and Mycro2 displayed good specificities in vitro, they showed only weak-to-moderate specificity for c-Myc-dependent transcription over transcription mediated by AP-1 family proteins, which also dimerize via leucine zippers. A follow-up screen using a focused library of pyrazolo[1,5-a]pyrimidines led to the discovery of the pyrazolo[1,5-fl]pyrimidine 1 (Mycro3), which inhibited c-Myc/Max dimerization and DNA binding with very good selectivity in vitro, and also showed good potency and selectivity at concentrations of 10-40 μM against c-Myc in cellular assays (A. Kiessling, A, et al., ChemMedChem 2 (2007), pp. 627-630.).

It can be reasoned that inhibitors of the DNA-protein interactions between intact c-Myc/Max dimers and their DNA recognition motif should not interfere with gene transcription repressed by c-Myc, but would still block c-Myc induced transcriptional activation. This distinction can be used to help selectively identify compounds having this mechanism of action. In a screen designed to identify compounds that particularly affect cells with high levels of c-Myc, a compound termed MYRA-A, was discovered, which was shown to inhibit Myc-regulated gene expression, oncogenic transformation, and to induce apoptosis in a Myc-dependent manner (H. Mo and M. Hennksson, Proc Natl Acad Sci USA 103 (2006), pp. 6344-6349.). In a subsequent study, the same group published an additional inhibitor of DNA binding of c-Myc/Max family members dubbed NSC308848 (Mo, H., et al. Cell Cycle 5 (2006), pp. 2191-2194.).

Hammoudeh, et al. (2009) identified multiple small molecule binding sites on c-Myc, facilitating use of drug design and/or virtual screening to identify additional c-Myc inhibitors.

Some exemplary small molecule c-Myc inhibitors of use in various embodiments of the invention are shown below. In certain embodiments of the invention analogs of any of these compounds are used.

10074-GS



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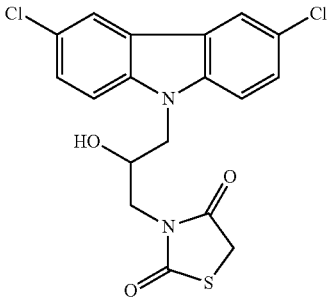
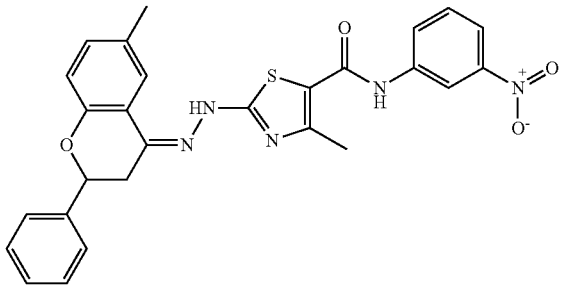
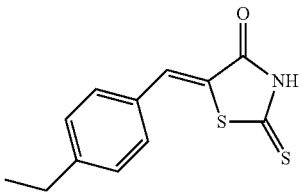
10074-A4



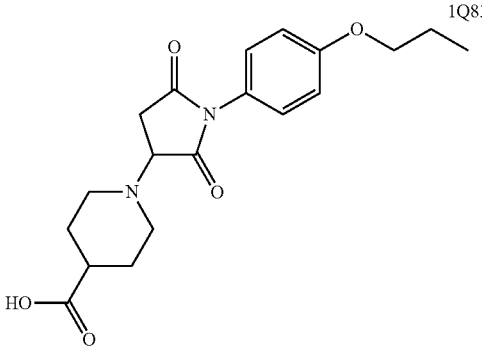
10050-C10

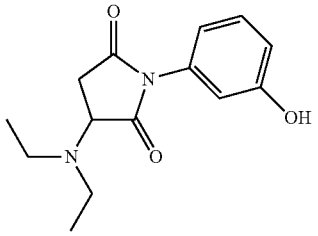


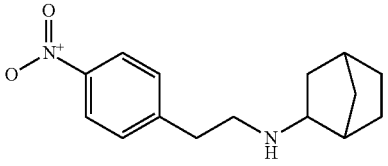
1 CSQSS~F4



1Q831-B8







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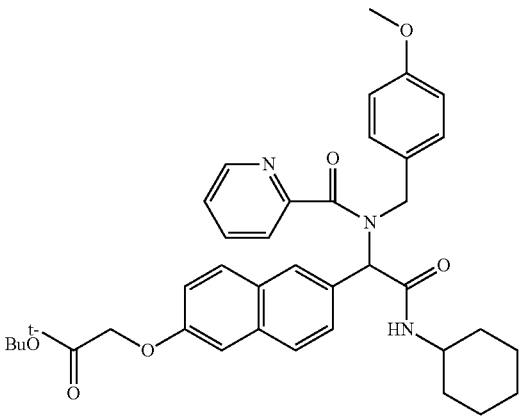
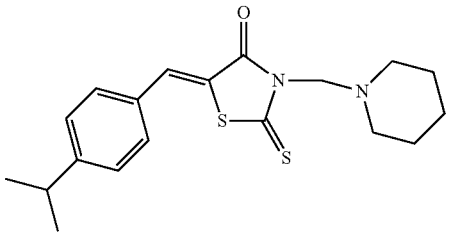
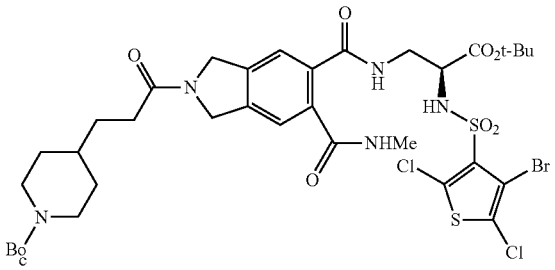
NY2267



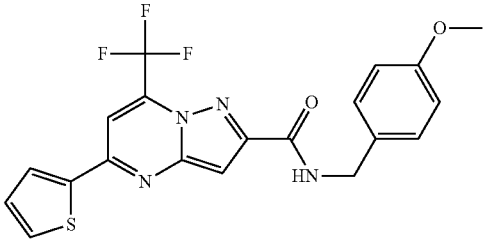
28RH-NCN-1



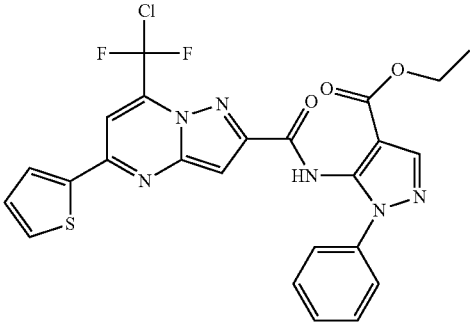
IIA6B17



Mycro 1



Mycro 3



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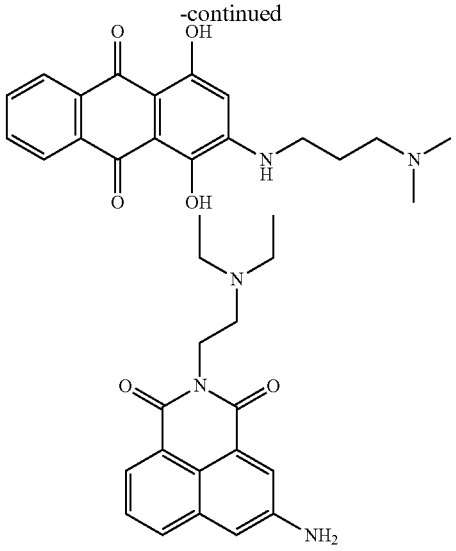

In some embodiments, a c-Myc inhibitor comprises an RNAi agent (e.g., an siRNA) or an antisense oligonucleotide that inhibits expression of c-Myc. In some embodiments a c-Myc inhibitor comprises an antibody or aptamer that specifically binds to c-Myc.

In some embodiments the agent promotes proteolysis of a polypeptide encoded by an oncogene in a cell (e.g., a tumor cell) exhibiting excessive levels of the cognate transcription factor and more transcriptional coactivator and chromatin regulator occupancy of the super-enhancer then the average single enhancer for the oncogene (e.g., an order of magnitude more). In some embodiments the agent promotes global proteolysis in cell-specific manner such that global proteolysis is only induced in those cells (e.g., tumor cells) exhibiting extremely high levels of the cognate transcription factor of the gene and transcriptional coactivator super-enhancer occupancy. In some embodiments the agent promotes proteolysis of a polypeptide encoded by one or more of a plurality of oncogenes in a cell in which cognate transcription factor levels are high and super-enhancers of the oncogene are occupied by more transcriptional coactivator than the average single enhancer of the oncogene.

The present invention contemplates the use of any agent that is capable of promoting proteolysis. In some embodiments the agent promotes global proteolysis of polypeptides encoded by the oncogenes. In some embodiments the agent promotes global proteolysis of polypeptides encoded by the oncogenes is promoted in cells that exhibit elevated cognate oncogenic transcription factors for the oncogene. In some embodiments the agent promotes global proteolysis of polypeptides is specific to tumor cells that possess oncogenes associated with super-enhancers. In some embodiments the agent promotes global proteolysis of polypeptides in cells that exhibit elevated cognate oncogenic transcription factors and excessive levels of transcriptional co-activator and/or chromatin regulator co-occupancy of super-enhancers and active transcription start sites.

In some embodiments the agent promotes global proteolysis of polypeptides by targeting the oncogene and its expression products for ubiquitin-dependent proteolysis. In some embodiments, the agent promotes global proteolysis of polypeptides by ubiquitin-dependent proteolysis by the proteasome. Ubiquitin-dependent proteolysis is a pathway used by eukaryotic cells for degrading cellular proteins. Protein

ubiquitination is catalyzed by the concerted actions of three classes of enzymes; the E1 ubiquitin-activating enzymes, the E2 ubiquitin-conjugating enzymes, and the E3 ubiquitin protein ligases (Hochstrasser, Annu Rev. Genet 30: 405-39, 1996). E1 and E2 are involved in the activation and transfer of ubiquitin, while the substrate specificity of the ubiquitin pathway is conferred by the E3 ubiquitin protein ligases. In some embodiments the agent comprises a ubiquitin protein ligase polypeptide. In some embodiments the agent is an E3 ubiquitin protein ligase polypeptide. In some embodiments the E3 ubiquitin protein ligase is an SCF polypeptide. In some embodiments the agent is a HECT polypeptide. In some embodiments the agent is a UBR1 polypeptide. In some embodiments the E3 ubiquitin protein ligase polypeptide is an F-box polypeptide (e.g., an F-box polypeptide which further comprises a WD domain). In some embodiments the F-box polypeptide is Cdc4p. In some embodiments the F-box polypeptide is Pop1p. In some embodiments the F-box polypeptide is Pop 2p. In some embodiments the F-box polypeptide is Grr1p. In some embodiments the F-box polypeptide is Met30p. In some embodiments the F-box polypeptide is HOSp. In some embodiments the F-box polypeptide is beta TrCPp. In some embodiments the F-box polypeptide is FWD1p. In some embodiments the F-box polypeptide is a polypeptide which is at least 70% identical to a contiguous polypeptide sequence of a polypeptide selected from the group consisting of SEQ ID Nos. 2, 4, 6, 8, 10, and 12 described in U.S. Pat. No. 7,223,556, which is incorporated herein by reference. In some embodiments the F-box polypeptide is at least 80% identical to a contiguous nucleic acid sequence of SEQ ID Nos. 1, 3, 5, 7, 9, and 11 described in U.S. Pat. No. 7,223,556, which is incorporated herein by reference.

In some embodiments the agent destabilizes RNA and/or proteins produced by the oncogene. In some embodiments an agent that destabilizes RNA is an agent that modulates nonsense-mediated RNA decay (NMD). Gardner discusses NMD implications for tumorigenesis (Gardner. Mol Cancer Res. 8; 295, 2010). In some embodiments an agent that modulates NMD is an agent that induces NMD of RNA transcripts of cognate oncogenic transcription factors, transcriptional coactivators, or chromatin regulators. In some embodiments an agent that modulates NMD is an agent that downregulates NMD that has been upregulated in a tumor. In some embodiments an agent that modulates NMD is an agent that inhibits Upf1. In some embodiments an agent that inhibits Upf1 is Pateamine A (PatA), as is described by Dang et al. (Dang et al. J Biol Chem. 284(35):23613-21, 2009).

In some embodiments the agent blocks mRNA splicing. In some embodiments an agent that blocks mRNA splicing interferes with alternative splicing. In some embodiments an agent that blocks mRNA splicing is a specific inhibitor of CDC2-like kinase isoforms 1 and 4 (CLK1/CLK4) known as KH-CB19, as is described in Fedorov et al. (Fedorov et al. Chem Biol. 18(1):67-76, 2011). In some embodiments an agent that interferes with alternative spicing is amiloride, as is described by Chang et al. PLos ONE. 6(6):e18643).

In some embodiments an agent that blocks mRNA splicing is an inhibitor of spliceosome catalysis. In some embodiments an agent that inhibits spliceosome catalysis is a 1,4-napthoquinones and/or a 1,4-heterocyclic quinone, non-limiting examples of which are described by Berg et al. (Berg et al. Mol Cell Biol. 32(7):1271-83, 2012). In some embodiments the splicing inhibitor comprises the benzothiazole-4, 7-dione, BN82685, which blocks the second of two transesterification splicing reactions, preventing the release of intron lariat and exon ligation (Berg et al. 2012). In an embodiment an agent that blocks mRNA splicing comprises 4μ8C, which blocks substrate access to an IRE1 active site and selectively inactivates Xpb1 splicing, as is described by Cross et al. (Cross et al. Proc Natl Acad Sci USA, Epub ahead of print on Feb. 6, 2012).

In some embodiments the agent inhibits translation of mRNA into protein. In some embodiments an agent that inhibits translation of mRNA into protein comprises a nucleoside 5'-monophosphate analog of the mRNA 5'-cap, for example, Barzynkiewics et al. describe nucleotide cap analogs of 7-methylguanosine 5'monophosphate (m7GMP) that acted as competitive inhibitors of capped mRNA translation, including analogs in which the 7-methyl moiety is substituted with 7-ethyl (e7), 7-propyl (p7), 7-isopropyl (ip7), 7-butyl (b7), 7-isobutyl (ib7), 7-cyclopentyl (cp7), 7-(carboxymethyl) (cm7), 7-benzyle (bn7), 7-(2-phenylethyl) [7-(2-PhEt)], and 7-(1-penylethyl) [7-(1-PhEt)]. (Darzynkiewics et al. 28(11):4771-8, 1989).

It should be appreciated that the various agents described herein can be used alone, or in combination with other agents described, for example, an agent that interferes with c-Myc enhancer-driven transcription of a plurality of Myc target genes as described in U.S. Application Ser. No. 61/621,897, the entirety of which is hereby incorporated by reference herein.

In some embodiments, an agent of the present invention is administered in combination with a cancer therapeutic agent. It should be appreciated that the combined administration of an agent of the present invention and a cancer therapeutic agent can be achieved by formulating the cancer therapeutic agent and agent in the same composition or by administering the cancer therapeutic agent and agent separately (e.g., before, after, or interspersed with doses or administration of the cancer therapeutic agent). In some embodiments, an agent of the present invention is administered to a patient undergoing conventional chemotherapy and/or radiotherapy. In some embodiments the cancer therapeutic agent is a chemotherapeutic agent. In some embodiments the cancer therapeutic agent is an immunotherapeutic agent. In some embodiments the cancer therapeutic agent is a radiotherapeutic agent.

Exemplary chemotherapeutic agents that can be administered in combination with the agents of the present invention (e.g., agents that disrupt the function of super-enhancers) include alkylating agents (e.g. cisplatin, carboplatin, oxaloplatin, mechlorethamine, cyclophosphamide, chorambucil, nitrosureas); anti-metabolites (e.g. methotrexate, pemetrexed, 6-mercaptopurine, dacarbazine, fludarabine, 5-fluorouracil, arabinosycytosine, capecitabine, gemcitabine, decitabine); plant alkaloids and terpenoids including vinca alkaloids (e.g. vincristine, vinblastine, vinorelbine), podophyllotoxin (e.g. etoposide, teniposide), taxanes (e.g. paclitaxel, docetaxel); topoisomerase inhibitors (e.g. notecan, topotecan, amasacrine, etoposide phosphate); antitumor antibiotics (dactinomycin, doxorubicin, epirubicin, and bleomycin); ribonucleotides reductase inhibitors; antimicrotubules agents; and retinoids. (See, e.g., Cancer: Principles and Practice of Oncology (V. T. DeVita, et al., eds., J.B. Lippincott Company, 9th ed., 2011; Brunton, L., et al. (eds.) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th Ed., McGraw Hill, 2010).

Exemplary immunotherapeutic agents include cytokines, such as, for example interleukin-1 (IL-I), IL-2, IL-4, IL-5, IL-13, IL-7, IL-10, IL-12, IL-I5, IL-18, CSF-GM, CSF-G, IFN-γ, IFN-α, TNF, TGF-β but not limited thereto.

In some embodiments an agent of the present invention can be linked or conjugated to a delivery vehicle, which may also contain cancer therapeutic. Suitable delivery vehicles include

liposomes (Hughes et al. Cancer Res 49(22):6214-20, 1989, which is hereby incorporated by reference in its entirety), nanoparticles (Farokhzad et al. Proc Nat'l Acad Sci USA 103(16):6315-20, 2006, which is hereby incorporated by reference in its entirety), biodegradable microspheres, microparticles, and collagen minipellets. The delivery vehicle can contain any of the agents and/or compositions of the present invention, as well as chemotherapeutic, radiotherapeutic, or immunotherapeutic agents described supra.

In some embodiments an agent of the present invention can be conjugated to a liposome delivery vehicle (Sofou and Sgouros, Exp Opin Drug Deliv. 5(2):189-204, 2008, which is hereby incorporated by reference in its entirety). Liposomes are vesicles comprised of one or more concentrically ordered lipid bilayers which encapsulate an aqueous phase. Suitable liposomal delivery vehicles are apparent to those skilled in the art. Different types of liposomes can be prepared according to Bangham et al. J. Mol. Biol. 13:238-52, 1965; U.S. Pat. No. 5,653,996 to Hsu; U.S. Pat. No. 5,643,599 to Lee et al.; U.S. Pat. No. 5,885,613 to Holland et al.; U.S. Pat. No. 5,631, 237 to Dzau & Kaneda; and U.S. Pat. No. 5,059,421 to Loughrey et al., which are hereby incorporated by reference in their entirety.

These liposomes can be produced such that they contain, in addition to the therapeutic agents of the present invention, other therapeutic agents, such as immunotherapeutic cytokines, which would then be released at the target site (e.g., Wolff et al., Biochim. Biophys. Acta. 802:259-73, 1984, which is hereby incorporated by reference in its entirety).

The present invention also contemplates a composition comprising an agent of the present invention and a pharmaceutically acceptable carrier, diluent, or excipient. Therapeutic formulations of the agents of the present invention can be prepared having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (REMINGTON'S PHARMACEUTICAL SCIENCES (A. Osol ed. 1980), which is hereby incorporated by reference in its entirety), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as acetate, Tris-phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; tonicifiers such as trehalose and sodium chloride; sugars such as sucrose, mannitol, trehalose or sorbitol; surfactant such as polysorbate; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG).

The active therapeutic ingredients of the pharmaceutical compositions alone or in combination with or linked to a cancer therapeutic agent or radiotherapeutic agent) can be entrapped in microcapsules prepared using coacervation techniques or by interfacial polymerization, e.g., hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (e.g., liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in REMINGTON'S PHARMACEUTICAL SCIENCES (A. Osol ed. 1980), which is hereby incorporated by reference in its entirety. In some embodiments the agents of the present invention can be conjugated to the microcapsule delivery vehicle to target the delivery of the therapeutic agent to the site of the cells exhibiting super-enhancer associated oncogenes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody or polypeptide, which matrices are in the form of shaped articles, e.g., films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides, copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT® (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−)-3-hydroxybutyric acid.

In some embodiments, an agent of the present invention can be provided with an enteric coating or otherwise protected from hydrolysis or low stomach pH. The therapeutically effective compositions containing the agents of the present invention are administered to a subject, in accordance with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

Other therapeutic regimens may be combined with the administration of the agents of the present invention. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Preferably such combined therapy results in a synergistic therapeutic effect. In some embodiments, a composition of the present invention is administered in combination with a therapy selected from the group consisting of chemotherapy, radiotherapy, proton therapy, surgery, and combinations thereof.

The composition can include any number of additional active ingredients which can act in concert to provide a therapeutic effect, (e.g., a synergistic therapeutic effect), such as a chemotherapeutic agent, a radiotherapeutic agent, a nutritional supplement (e.g. vitamins), an antioxidant, and combinations thereof.

An "effective amount" or "effective dose" of an agent (or composition containing such agent) generally refers to the amount sufficient to achieve a desired biological and/or pharmacological effect, e.g., when contacted with a cell in vitro or administered to a subject according to a selected administration form, route, and/or schedule. As will be appreciated by those of ordinary skill in the art, the absolute amount of a particular agent or composition that is effective may vary depending on such factors as the desired biological or pharmacological endpoint, the agent to be delivered, the target tissue, etc. Those of ordinary skill in the art will further understand that an "effective amount" may be contacted with cells or administered in a single dose, or through use of multiple doses, in various embodiments. It will be understood

that agents, compounds, and compositions herein may be employed in an amount effective to achieve a desired biological and/or therapeutic effect.

In certain aspects, the present invention relates to a method of treating a proliferative disorder in a patient in need of such treatment, said proliferative disorder characterized by an oncogene-associated super-enhancer occupied by more Mediator or BRD4 than an average single enhancer, comprising administering to the patient an effective amount of an agent that disrupts the function of the oncogene-associated super-enhancer, thereby selectively inhibiting proliferation of the oncogene in the patient.

It should be apparent to those skilled in the art that any of the compounds or agents described above can be employed in the method of treating the proliferative disorder to achieve the desired result of disrupting the function of the super-enhancer. The present invention contemplates the treatment of any proliferative disorder (e.g., cancer) that is characterized by an oncogene-associated super-enhancer. In some embodiments, the proliferative disorder to be treated is a hematological malignancy. In some embodiments, the proliferative disorder to be treated is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL), Mantle cell lymphoma, B-cell lymphoma, acute lymphoblastic T cell leukemia (T-ALL), acute promyelocytic leukemia, and multiple myeloma. In some embodiments, the proliferative disorder is a non-hematological malignancy.

In certain exemplary embodiments, the agent is a BRD4 inhibitor, for example, small molecule JQ1 or iBET.

In some aspects, the present invention relates to a method of treating multiple myeloma involving an IGH-MYC locus that results in aberrant expression of oncogene c-Myc, comprising administering to a patient in need of such treatment an effective amount of an agent that decreases occupancy levels of BRD4 and MED1 at a super-enhancer associated with the IGH-MYC locus, wherein decreased occupancy levels of BRD4 and MED1 at the super-enhancer disrupt function of the super-enhancer thereby decreasing aberrant expression of oncogene c-Myc such that the multiple myeloma is treated. In some embodiments, the agent is a BRD4 inhibitor, for example, JQ1 or iBET.

In some aspects, the present invention relates to a method of identifying an agent that disrupts a super-enhancer associated with a gene, comprising: (a) providing a cell or cell free system comprising a super-enhancer, or functional fragment and/or variant thereof, and an associated gene, e.g., a reporter gene; (b) contacting the cell with a test agent, e.g., under conditions suitable for the super-enhancer to drive expression of the associated gene, e.g., to drive expression at a preselected level, e.g., a high level; (c) and measuring the level of expression of the associated gene.

In an embodiment decreased expression of the associated gene in the presence of the test agent indicates that the test agent is as an agent that disrupts the super-enhancer associated with the gene.

In an embodiment the method comprises transfecting a cell with a super-enhancer and the associated gene under conditions suitable for the super-enhancer to drive high levels of expression of the associated gene.

In an embodiment the method comprises comparing the level of expression with a reference, e.g., expression in a similar system not contacted with the test agent.

In an embodiment the method comprises confirming disruption of the super-enhancer, or functional fragment and/or variant thereof, e.g., by analysis of the presence of one or more super-enhancer component.

In an embodiment the method is first performed in a cell-free system and repeated in cell preparation, e.g., a cultured cell.

In an embodiment the method is first performed in a cell-free system or a cell preparation, e.g., a cultured cell, and repeated in an animal.

In an embodiment the super-enhancer is associated with a gene that is expressed in a disease state cell, e.g., a cancer cell.

In an embodiment the method comprises memorializing the results.

A wide variety of test agents can be used in the methods. For example, a test agent can be a small molecule, polypeptide, peptide, nucleic acid, oligonucleotide, lipid, carbohydrate, or hybrid molecule. Compounds can be obtained from natural sources or produced synthetically. Compounds can be at least partially pure or may be present in extracts or other types of mixtures. Extracts or fractions thereof can be produced from, e.g., plants, animals, microorganisms, marine organisms, fermentation broths (e.g., soil, bacterial or fungal fermentation broths), etc. In some embodiments, a compound collection ("library") is tested. The library may comprise, e.g., between 100 and 500,000 compounds, or more. Compounds are often arrayed in multwell plates. They can be dissolved in a solvent (e.g., DMSO) or provided in dry form, e.g., as a powder or solid. Collections of synthetic, semi-synthetic, and/or naturally occurring compounds can be tested. Compound libraries can comprise structurally related, structurally diverse, or structurally unrelated compounds. Compounds may be artificial (having a structure invented by man and not found in nature) or naturally occurring. In some embodiments a library comprises at least some compounds that have been identified as "hits" or "leads" in other drug discovery programs and/or derivatives thereof. A compound library can comprise natural products and/or compounds generated using non-directed or directed synthetic organic chemistry. Often a compound library is a small molecule library. Other libraries of interest include peptide or peptoid libraries, cDNA libraries, and oligonucleotide libraries. A library can be focused (e.g., composed primarily of compounds having the same core structure, derived from the same precursor, or having at least one biochemical activity in common).

Compound libraries are available from a number of commercial vendors such as Tocris BioScience, Nanosyn, BioFocus, and from government entities. For example, the Molecular Libraries Small Molecule Repository (MLSMR), a component of the U.S. National Institutes of Health (NIH) Molecular Libraries Program is designed to identify, acquire, maintain, and distribute a collection of >300,000 chemically diverse compounds with known and unknown biological activities for use, e.g., in high-throughput screening (HTS) assays (see https://mli.nih.gov/mli/). The NIH Clinical Collection (NCC) is a plated array of approximately 450 small molecules that have a history of use in human clinical trials. These compounds are highly drug-like with known safety profiles. The NCC collection is arrayed in six 96-well plates. 50 μl of each compound is supplied, as an approximately 10 mM solution in 100% DMSO. In some embodiments, a collection of compounds comprising "approved human drugs" is tested. An "approved human drug" is a compound that has been approved for use in treating humans by a government regulatory agency such as the US Food and Drug Administration, European Medicines Evaluation Agency, or a similar agency responsible for evaluating at least the safety of thera-

peutic agents prior to allowing them to be marketed. The test agent may be, e.g., an antineoplastic, antibacterial, antiviral, antifungal, antiprotozoal, antiparasitic, antidepressant, antipsychotic, anesthetic, antianginal, antihypertensive, antiarrhythmic, antiinflammatory, analgesic, antithrombotic, antiemetic, immunomodulator, antidiabetic, lipid- or cholesterol-lowering (e.g., statin), anticonvulsant, anticoagulant, antianxiety, hypnotic (sleep-inducing), hormonal, or antihormonal drug, etc. In some embodiments, a compound is one that has undergone at least some preclinical or clinical development or has been determined or predicted to have "drug-like" properties. For example, the test agent may have completed a Phase I trial or at least a preclinical study in non-human animals and shown evidence of safety and tolerability. In some embodiments, a test agent is substantially non-toxic to cells of an organism to which the compound may be administered or cells in which the compound may be tested, at the concentration to be used or, in some embodiments, at concentrations up to 10-fold, 100-fold, or 1,000-fold higher than the concentration to be used. For example, there may be no statistically significant adverse effect on cell viability and/or proliferation, or the reduction in viability or proliferation can be no more than 1%, 5%, or 10% in various embodiments.

In various embodiments of any aspect herein pertaining to screening methods (e.g., methods of identifying agents), the screen may be performed using a single test agent or multiple test agents in a given reaction vessel. In various embodiments the number of reaction vessels and/or test agents is at least 10; 100; 1000; 10,000; 100,000, or more. In some embodiments of any aspect herein pertaining at least in part to screening methods (e.g., methods of identifying agents) a high throughput screen (HTS) is performed. High throughput screens often involve testing large numbers of test agents with high efficiency, e.g., in parallel. For example, tens or hundreds of thousands of agents may be routinely screened in short periods of time, e.g., hours to days. Such screening is often performed in multiwell plates (sometimes referred to as microwell or microtiter plates or microplates) containing, e.g., 96, 384, 1536, 3456, or more wells or other vessels in which multiple physically separated depressions, wells, cavities, or areas (collectively "wells") are present in or on a substrate. Different test agent(s) may be present in or added to the different wells. It will be understood that some wells may be empty, may comprise replicates, or may contain control agents or vehicle. High throughput screens may involve use of automation, e.g., for liquid handling, imaging, and/or data acquisition or processing, etc. In some embodiments an integrated robot system comprising one or more robots transports assay-microplates from station to station for, e.g., addition, mixing, and/or incubation of assay constituents (e.g., test agent, target, substrate) and, in some embodiments, readout or detection. A HTS system may prepare, incubate, and analyze many plates simultaneously. Certain general principles and techniques that may be applied in embodiments of a HTS are described in Macarrón R & Hertzberg R P. Design and implementation of high-throughput screening assays. Methods Mol Biol., 565:1-32, 2009 and/or An W F & Tolliday N J., Introduction: cell-based assays for high-throughput screening. Methods Mol Biol. 486:1-12, 2009, and/or references in either of these. Exemplary methods are also disclosed in High Throughput Screening: Methods and Protocols (Methods in Molecular Biology) by William P. Janzen (2002) and High-Throughput Screening in Drug Discovery (Methods and Principles in Medicinal Chemistry) (2006) by Jorg Hüser. Test agent(s) showing an activity of interest (sometimes termed "hits") may be retested and/or, optionally (e.g., depending at least in part on results of retesting) selected for further testing, development, or use. In some embodiments one or more structural analogs of a hit is synthesized. Such analogs may, for example, comprise substitution of one or more functional groups or heteroatoms present in the hit by a different functional group or heteroatom or substituting a heteroatom or functional group present in place of a hydrogen in the hit, etc. In some embodiments one or more such analog(s) are then tested for a property or activity of interest (e.g., ability to disrupt a super-enhancer associated with an oncogene or disease related gene).

Positive and/or negative controls may be used in any of the screens. An appropriate positive or negative control can be selected based at least in part on the assay. A negative control may be to perform the assay in the absence of a test agent.

In some embodiments, information derived from sequence analysis, mutational analysis, and/or structural analysis is used in the identification of a modulator, e.g., an agent that interferes with transcriptional coactivator or BRD4 co-occupancy of super-enhancers and active transcription start sites. For example, in some embodiments a structure (e.g., a two-dimensional or three-dimensional structure) of a target, e.g., a TF, generated at least in part using, e.g., nuclear magnetic resonance, homology modeling, and/or X-ray crystallography is used. In some embodiments a structure obtained with a ligand (e.g., an inhibitor) bound to the target may be used. In some embodiments a computer-aided computational approach sometimes referred to as "virtual screening" is used in the identification of candidate modulators. Structures of compounds, e.g., small molecules may be screened for ability to bind to a region (e.g., a "pocket") accessible to the compound. The region may be any region accessible to the compound, e.g., a concave region on the surface or a cleft or a region involved in dimerization. A variety of docking and pharmacophore-based algorithms are known in the art, and computer programs implementing such algorithms are available. Commonly used programs include Gold, Dock, Glide, FlexX, Fred, and LigandFit (including the most recent releases thereof). See, e.g., Ghosh, S., et al., Current Opinion in Chemical Biology, 10(3): 194-2-2, 2006; McInnes C., Current Opinion in Chemical Biology; 11(5): 494-502, 2007, and references in either of the foregoing articles, which are incorporated herein by reference. In some embodiments a virtual screening algorithm may involve two major phases: searching (also called "docking") and scoring. During the first phase, the program automatically generates a set of candidate complexes of two molecules (test compound and target molecule) and determines the energy of interaction of the candidate complexes. The scoring phase assigns scores to the candidate complexes and selects a structure that displays favorable interactions based at least in part on the energy. To perform virtual screening, this process may be repeated with a large number of test compounds to identify those that, for example, display the most favorable interactions with the target. In some embodiments, low-energy binding modes of a small molecule within an active site or possible active site or other target region are identified. In some embodiments a compound capable of docking at a site where mutations are known to inhibit activity of the target is identified. Variations may include the use of rigid or flexible docking algorithms and/or including the potential binding of water molecules. In some embodiments the three-dimensional structure of an enzyme's active site may be used to identify potential inhibitors. Agent(s) that have the potential to bind in or near an active site may be identified. These predictions may then be tested using the actual compound. A new inhibitor thus identified may then be used to obtain a structure of the enzyme in

an inhibitor/enzyme complex to show how the molecule is binding to the active site. Further changes may be made to the inhibitor, e.g., to try to improve binding. This cycle may be repeated until an inhibitor of sufficient predicted or actual potency (e.g., a desired potency for therapeutic purposes) is identified. Numerous small molecule structures are available and can be used for virtual screening. A collection of compound structures may sometimes referred to as a "virtual library". For example, ZINC is a publicly available database containing structures of millions of commercially available compounds that can be used for virtual screening (http://zinc.docking.org/; Shoichet, J. Chem. Inf. Model., 45(1):177-82, 2005). A database containing about 250,000 small molecule structures is available on the National Cancer Institute (U.S.) website (at http://129.43.27.140/ncidb2/). In some embodiments multiple small molecules may be screened, e.g., up to 50,000; 100,000; 250,000; 500,000, or up to 1 million, 2 million, 5 million, 10 million, or more. Compounds can be scored and, optionally, ranked by their potential to bind to a target. Compounds identified in virtual screens can be tested in cell-free or cell-based assays or in animal models to confirm their ability to inhibit activity of a target molecule, their ability to activate a target molecule, and/or to assess their biological and/or pharmacological activity. Computational approaches may be used to predict one or more physicochemical, pharmacokinetic and/or pharmacodynamic properties of compounds identified in a physical or virtual screen. Such information may be used, e.g., to select one or more hits for, e.g., further testing, development, or use. For example, small molecules having characteristics typical of "drug-like" molecules may be selected and/or small molecules having one or more undesired characteristics may be avoided.

In some aspects of any screening and/or characterization methods, test agents are contacted with test cells (and optionally control cells) or used in cell-free assays at a predetermined concentration. In some embodiment the concentration is about up to 1 nM. In some embodiments the concentration is between about 1 nM and about 100 nM. In some embodiments the concentration is between about 100 nM and about 10 μM. In some embodiments the concentration is at or above 10 μM e.g., between 10 μM and 100 μM. Following incubation for an appropriate time, optionally a predetermined time, the effect of compounds or composition on a parameter of interest in the test cells is determined by an appropriate method known to one of ordinary skill in the art, e.g., as described herein. Cells can be contacted with compounds for various periods of time. In certain embodiments cells are contacted for between 12 hours and 20 days, e.g., for between 1 and 10 days, for between 2 and 5 days, or any intervening range or particular value. Cells can be contacted transiently or continuously. If desired, the compound can be removed prior to assessing the effect on the cells.

One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The details of the description and the examples herein are representative of certain embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention. It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention provides all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. It is contemplated that all embodiments described herein are applicable to all different aspects of the invention where appropriate. It is also contemplated that any of the embodiments or aspects can be freely combined with one or more other such embodiments or aspects whenever appropriate. Where elements are presented as lists, e.g., in Markush group or similar format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. For example, any one or more nucleic acids, polypeptides, cells, species or types of organism, disorders, subjects, or combinations thereof, can be excluded.

Where the claims or description relate to a composition of matter, e.g., a nucleic acid, polypeptide, cell, or non-human transgenic animal, it is to be understood that methods of making or using the composition of matter according to any of the methods disclosed herein, and methods of using the composition of matter for any of the purposes disclosed herein are aspects of the invention, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where the claims or description relate to a method, e.g., it is to be understood that methods of making compositions useful for performing the method, and products produced according to the method, are aspects of the invention, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

Where ranges are given herein, the invention includes embodiments in which the endpoints are included, embodiments in which both endpoints are excluded, and embodiments in which one endpoint is included and the other is excluded. It should be assumed that both endpoints are included unless indicated otherwise. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly

dictates otherwise. It is also understood that where a series of numerical values is stated herein, the invention includes embodiments that relate analogously to any intervening value or range defined by any two values in the series, and that the lowest value may be taken as a minimum and the greatest value may be taken as a maximum. Numerical values, as used herein, include values expressed as percentages. For any embodiment of the invention in which a numerical value is prefaced by "about" or "approximately", the invention includes an embodiment in which the exact value is recited. For any embodiment of the invention in which a numerical value is not prefaced by "about" or "approximately", the invention includes an embodiment in which the value is prefaced by "about" or "approximately". "Approximately" or "about" generally includes numbers that fall within a range of 1% or in some embodiments within a range of 5% of a number or in some embodiments within a range of 10% of a number in either direction (greater than or less than the number) unless otherwise stated or otherwise evident from the context (except where such number would impermissibly exceed 100% of a possible value). It should be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one act, the order of the acts of the method is not necessarily limited to the order in which the acts of the method are recited, but the invention includes embodiments in which the order is so limited. It should also be understood that unless otherwise indicated or evident from the context, any product or composition described herein may be considered "isolated".

EXAMPLES

Example 1

Master Transcription Factors and Mediator Establish Super-Enhancers at Key Cell Identity Genes

Introduction

Transcription factors typically regulate gene expression by binding cis-acting regulatory elements known as enhancers and recruiting coactivators and RNA Polymerase II (RNA Pol II) to target genes (Ong and Corces, 2011), Transcription factor-bound enhancers interact with target gene promoters via DNA looping events facilitated by the Mediator co-activator complex and cohesin (Kagey et al., 2010). Between 400,000 and 1.4 million putative enhancers have been identified in the mammalian genome (Bernstein et al., 2012; Thurman et al., 2012). In any one cell type, the number of active enhancers is estimated to be in the thousands and enhancer activity is largely cell-type specific (Bernstein et al., 2012; Shen et al., 2012; Yip et al., 2012). Whereas most genes are transcriptionally active in multiple cell types, enhancers tend to be active only in specific lineages (Shen et al., 2012). These data suggest that much of the transcriptional control of mammalian development is due to the diverse activity of enhancers that control cell type specific patterns of gene expression.

In embryonic stem cells (ESCs), control of the gene expression program that establishes and maintains ESC state is dependent on a remarkably small number of master transcription factors (Young, 2011). These transcription factors, which include Oct4, Sox2 and Nanog (OSN), bind to approximately 7,000 enhancers together with the Mediator coactivator complex (Kagey et al., 2010). The Mediator complex facilitates the ability of enhancer-bound transcription factors to recruit RNA Pol II to the promoters of target genes (Malik and Roeder, 2010) and is essential for maintenance of ESC state and early embryonic development (Kagey et al., 2010). Reduced levels of either Oct4 or Mediator have a very similar effect on the ESC gene expression program and cause the same rapid loss of ESC identity (Kagey et al., 2010).

It is striking that ESC maintenance is highly sensitive to perturbations in the levels of Mediator (Kagey et al., 2010). To understand the reasons underlying this hypersensitivity, we investigated enhancers bound by Mediator in these cells. We identified approximately 200 genomic regions that contained tightly spaced clusters of enhancers spanning extraordinarily large domains. These "super-enhancers" were occupied by an order of magnitude more Mediator than the average enhancer, and were associated with the key cell-type specific ESC genes. These enhancers also conferred stronger enhancer activity relative to the average enhancer, suggesting these elements drive gene expression programs and cell state. During ESC differentiation, the ESC super-enhancers were rapidly lost and new super-enhancers were formed at genes key to the differentiated cell type. Additional cell types were found to have super-enhancers associated with highly expressed and cell-type specific genes. These results argue that super-enhancers drive genes essential for cell identity in multiple cell types and that these elements are especially sensitive to perturbations involved in dynamic changes in cell state.

Results

Large Genomic Domains Occupied by Mediator in ESCs

Previous studies have shown that co-occupancy of sites by the Oct4, Sox2 and Nanog transcription factors is highly predictive of enhancer activity (Chen et al., 2008). We generated ChIP-Seq data for Oct4, Sox2, Nanog (OSN) in murine ESCs and identified 6,343 regions that were bound by all three transcription factors. The Mediator co-activator complex has been previously shown to interact with the enhancer-bound transcription factors and facilitate recruitment of the transcription apparatus to active gene promoters (Malik and Roeder, 2010). Analysis of the 6,343 OSN regions confirmed the presence of Mediator, including regions surrounding the Klf4 gene (FIG. **1**A). Therefore, we defined the 6,343 regions bound by OSN as ESC enhancers.

Closer inspection of the 6,343 ESC enhancers revealed a surprising feature: some ESC enhancers are occupied by extremely high levels of Mediator (FIG. **1**B). Global analysis of the 6,343 ESC enhancers confirmed the distribution of Mediator occupancy across this set of regions is not evenly distributed (FIG. **1**C). Instead, there is a distribution of occupancy that indicates these regions fall into two distinct classes, with one class containing an exceptional amount of Mediator proteins (FIG. **1**C). Further analysis of this small subset (211) of regions revealed that, on average, they contained 27 times more Mediator proteins compared to the remaining 6,132 enhancers (FIG. **1**D). Additionally, on average these regions covered larger genomic distances (5.2 kb) compared to the remaining enhancers (469 bp) (FIG. **1**D). Thus, these ~200 regions, which we call "super-enhancers", are occupied by at least an order of magnitude more Mediator relative to the mean, and typically span DNA domains at least an order of magnitude larger.

Many genome wide enhancer mapping efforts utilize histone marks and regulatory proteins as surrogates for enhancers (Bernstein et al., 2012; Shen et al., 2012). Further characterization of the super-enhancers revealed that these regions are also occupied by other enhancer-associated modifications and proteins, including H3K27ac, a histone modification commonly found at enhancers and used to predict regions of enhancers activity (Creyghton et al., 2010; Rada-Iglesias et al., 2011). Interestingly, H3K27Ac failed to reveal the striking disparity noted for OSN-Mediator bound super-

enhancers. Thus, Mediator ChIP-Seq data is superior to surrogate data from histone modifications for identifying super-enhancers in ESCs.

Super-enhancers are Associated with Key ESC Genes

Most studies have assigned enhancers to putative target genes by using the proximity of enhancers and target genes. Recent work has identified topological domains associated with transcriptional control in the ESC genome using high throughputs chromatin conformation capture data (Hi-C) (Dixon et al., 2012). We therefore used proximity of enhancer elements and genes to facilitate mapping of ESC enhancers to promoters, and further used Hi-C to additionally assign enhancers to promoters of genes that were greater than 40 kb away. Previous studies using chromatin configuration capture (3C) have shown that, at an enhancer element brought into close proximity to a promoter region by DNA looping, the Mediator ChIP-Seq signals are similar at both regions (Kagey et al., 2010). We therefore required that enhancer-promoter interaction candidates have similar levels of Mediator. The assignments of super-enhancers to promoters identified 192 genes, with a further ~5,300 assigned by Hi-C. For three of these genes, the proximity between portions of the super-enhancer and the target promoter were previously established using 3C (Kagey et al., 2010).

A global RNA sequencing (RNA-Seq) analysis of the genes assigned to ESC enhancers confirmed that these genes were expressed at very high levels compared to other genes in ESCs (FIG. **2**A). Further examination of this set of genes, however, revealed a striking difference: the super-enhancer-associated genes were expressed at higher levels compared to those neighboring the remaining enhancers (FIG. **2**B,C). Compared to the average expression levels of genes near the median enhancer (1.84 RPKM), genes associated with super-enhancers were expressed 6-times higher (FIG. **2**A). These results suggest super-enhancers are associated with the most highly expressed genes compared to other enhancers.

We next determined if these highly expressed genes were important for ESC identity. In contrast to the other highly expressed genes that were found near the 6,132 enhancers, including house-keeping genes, super-enhancer-associated genes are critical for ESC maintenance and reprogramming. Super-enhancers were directly associated with many genes previously shown to play important roles in ESC identity, including Esrrb (Ivanova et al., 2006; Zhang et al., 2008); Tbx3 (Ivanova et al., 2006; Niwa et al., 2009); and the mir290-295 microRNA gene cluster (Lichner et al., 2011; Marson et al., 2008; Zovoilis et al., 2009). Remarkably, the super-enhancer-associated genes included those encoding the ESC master transcription factors Oct4, Sox2 and Nanog (FIG. **2**D). These three transcription factors are known to auto-regulate their expression through promoter binding, forming an interconnected auto-regulatory loop. This form of auto-regulation is a core feature of the ESC transcriptional regulatory circuitry (Boyer et al., 2005), whose establishment is likely key to reprogramming of various cells into iPS cells (Jaenisch and Young, 2008). Small portions of the super-enhancers associated with these genes have previously been shown to have enhancer activity in reporter assays (Chen et al., 2008) and to participate in enhancer-promoter looping at the Oct4 and Nanog genes (Kagey et al., 2010). Thus, the genes encoding the master transcription factors are themselves under the control of super-enhancers. Overall these results support a model that super-enhancers associate with highly expressed and highly cell-type specific genes that include key drivers of ESC identity.

Super-Enhancers Confer Strong Enhancer Activity

One striking feature of the super-enhancers is that they contain multiple, highly enriched regions of Mediator compared to average enhancers that typically consist of a single peak of the coactivator (FIG. **3**A). DNA sequence analysis confirmed that super-enhancers contained more OSN binding motifs than do median enhancers (FIG. **3**A). To test whether these super-enhancers confer stronger enhancer activity than median enhancers, we cloned 3 kb regions of super-enhancers and median enhancers into luciferase reporter constructs that were subsequently transfected into ESCs. We found that on average, super-enhancers drove 16 times more luciferase expression than median enhancers (FIG. **3**B). Since a super-enhancer contained more Mediator occupancy compared to a median enhancer, these results suggested that clusters of enhancers may display higher enhancer activity in ESCs. To test this model, we generated an artificial super-enhancer by oligomerizing the distal median enhancer of the Sgk1 gene. As a single median enhancer, this region displayed low luciferase activity (FIG. **3**B). Remarkably, the dimeric Sgk1 enhancer exhibited 2-times higher activity, while the tetrameric Sgk1 enhancer exhibited 3-times higher enhancer activity compared to the single Sgk1 enhancer driving luciferase expression in ESCs (FIG. **3**C). These results suggest that super-enhancers can be formed by clusters of enhancers, that they have higher activity than median enhancers, and are sufficient to drive high expression of key, cell type-specific genes required to maintain ESC identity.

Rapid Loss of ESC Super-enhancers During ESC Differentiation

If super-enhancers play key roles in transcriptional control of cell identity, then differentiation of ESCs should lead to loss of ESC super-enhancers. To test this notion, we stimulated ESCs to differentiate into a trophectoderm lineage by shutting down Oct4 transcription (FIG. **4**A)(Niwa et al., 2000). Loss of Oct4 results in cellular differentiation, loss of expression of Oct4 target genes, and upregulation of the trophectoderm master regulator transcription factor Cdx2 (Deb et al., 2006; Niwa et al., 2005; Strumpf et al., 2005; Wang et al., 2010).

The fate of ESC super-enhancers during differentiation was examined by profiling global levels of Mediator using ChIP-PCR (FIG. **4**B). All seven of the super-enhancers tested that were occupied by OSN and Mediator in ESCs had at least two-fold lower levels of Mediator proteins upon differentiation (FIG. **4**B). On average, the tested super-enhancers had 68% lower levels of Mediator upon ESC differentiation compared to control ESCs (FIG. **4**B). This included the super-enhancers to the key ESC genes Oct4/Pot5f1 and Sox2 (FIG. **4**B). In contrast, four of the five typical enhancers that were tested retained high levels of Mediator compared to super-enhancers upon ESC differentiation (FIG. **4**B). Surprisingly, only one of the typical enhancers tested had at least two-fold lower levels of Mediator upon differentiation (FIG. **4**B). On average, median enhancers had only 14% lower levels of Mediator upon differentiation compared to control ESCs (FIG. **4**B). Together, these results are consistent with the model that super-enhancers play key roles in establishing and maintaining cell state, and that these enhancer elements are sensitive to perturbations that accompany the dynamic changes in cell state during differentiation.

Super-Enhancers are Found in Multiple Cell Types and are Cell-Type Specific

The identification of both ESC and trophectoderm lineage super-enhancers suggest that super-enhancers may be a common feature of mammalian cells. Accordingly, in any given cell type, super-enhancer associated genes are likely to play prominent roles in establishing and maintaining cell identity.

Further, the pattern of super-enhancers in any given cell type is likely to be cell-type specific.

To test these predictions, we profiled Mediator levels and master transcription factor Pu.1 in pro-B cells using ChiP-Seq. Mediator occupancy highly correlated with occupancy of Pu.1 at promoter distal sites (FIG. **5**A, B). Of the 13,303 sites bound by Pu.1 in pro-B cells, 79% were co-occupied by Mediator. Using similar criteria as in ESCs, 392 super-enhancers were identified in pro-B cells, and exhibited extremely high levels of Mediator occupancy (FIG. **5**B,C). On average, the pro-B super-enhancers contained 31 times more Mediator proteins compared to the remaining 12,911 enhancers, and covered larger genomic distances (15.4 kb) compared to the remaining enhancers (422 bp). These findings support the conclusion that super-enhancers are a general feature of mammalian cells.

Genes associated with super-enhancers in pro-B cells were previously shown to be important for pro-B cell development, supporting the model that super-enhancers drive expression of target genes critical for cellular identity. Among the 355 super-enhancer-associated genes that are highly expressed in pro-B cells included many genes previously shown to play important roles in B cell development, including Pax5; Rag2; VpreB1 and VpreB2. We next determined if super-enhancers and their associated genes are cell-type specific by comparing ESC and pro-B cell super-enhancers and their target genes (FIG. **5**D,E). The set of super-enhancers showed minimal overlap between ESCs and pro-B cells (FIG. **5**D). Of the 211 ESC super-enhancers, only 9 regions (2%) overlapped with the pro-B cell super-enhancers (FIG. **5**D). Furthermore, the super-enhancer-associated genes exhibited highly cell-type specific patterns of expression (FIG. **5**D). Of the 192 genes neighboring super-enhancers in ESCs, only 15 (8%) were associated with super-enhancers in pro-B cells (FIG. **5**E). These results suggest that super-enhancers are likely to be a general feature of most cell types and are likely to drive the expression of genes controlling cellular identity.

Discussion

We have identified in multiple cell types the existence of super-enhancers. Super-enhancers are enhancers bound by master regulator transcription factors that contain disproportionately high levels of the Mediator co-activator complex. Mediator levels are likely to be rate limiting for enhancer mediated transcription and as such, the disparity in Mediator levels at super-enhancers potentially represents an important hierarchical stratification of enhancers. Indeed, in multiple cell types, super-enhancers associate with known genes essential for cell identity and globally are likely to be the drivers of key cell identity controlling genes.

The observation of super-enhancers also suggests the complexity of cis-regulating elements can be significantly reduced. Although somewhere between hundreds of thousand and millions of enhancers are likely to exist in the mammalian genome, in any given cell type only a few hundred super-enhancers are likely to drive the expression of genes that establish cellular identity. In many cell types, small subsets of transcriptionally active genes have been identified through genetic screens as essential for cellular identity. However an analogous appreciation does not exist for enhancers in any given cell types. The characteristic features of super-enhancers strongly suggest that they may be among the most essential enhancers in any given cell type.

Lastly, the ability of super-enhancers to drive expression of key cell identity genes suggest that mutations to super-enhancers may potentially lead to disease and developmental defect. Indeed, recent evidence from the ENCODE consortium revealed that the majority of disease associated SNPs occur in regulatory regions (Bernstein et al., 2012; Schaub et al., 2012). It is easy to imagine that loss of a super-enhancer through genetic deletions could lead to developmental defects through the inability to fully establish cellular identity. Conversely, translocation of a super-enhancer could result in aberrant gene regulation. Example 2 below provides evidence that super-enhancers associate with key cancer dependency genes, including c-Myc via the translocated IgH super-enhancer in Multiple Myeloma.

The association of super-enhancers with key cell identity genes as well as cancer dependency genes argues that super-enhancers are important and essential components of cellular identity. Given super-enhancers reflect the occupancy of master regulator transcription factors in a given cell type, identification of super-enhancers in any cell type could potentially facilitate the mapping of the core transcriptional circuitry. In disease cells, super-enhancers have the potential to act as powerful biomarkers, identifiers of drug target candidates, and can potentially they themselves be drugged via targeting of Mediator and other enhancer bound components. More importantly, the characterization of super-enhancers implores a departure from a gene centric view of the genome, and instead supports an appreciation that regulatory control regions found in intergenic DNA may represent key features in the blueprints of mammalian development and disease.

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Example 2

Selective Inhibition of Tumor Oncogenes by Disruption of Super-Enhancers

Introduction

Inhibitors of chromatin regulators are gaining interest as therapeutic agents for cancer because of their ability to specifically repress key oncogenic drivers in many tumor types. A major challenge in cancer therapeutics has been the direct pharmacologic inhibition of oncogenic transcription factors such as c-MYC. MYC is one of the most commonly amplified oncogenes in cancer, but lacks clear ligand-binding domains, rendering it difficult to target by small molecule inhibitors (Nair and Burley, 2003). However, several recent studies have shown that inhibition of chromatin regulators, such as the bromodomain protein BRD4, may represent an alternate avenue for selectively targeting these key oncogenic drivers. It is not yet known how inhibition of a general transcriptional regulator can exert a specific effect on a small number of genes. Understanding this concept will aid the development and selection of drugs in treating many cancers.

BRD4 was first identified as an interaction partner of the murine Mediator coactivator complex, and has subsequently been shown to associate with this transcription complex in a variety of human cells (Dawson et al., 2011; Jiang et al., 1998; Wu et al., 2003). BRD4 is also involved in the control of transcriptional elongation through its association with the positive transcription elongation factor, P-TEFb (Jang et al., 2005; Yang et al., 2005). In addition, bromodomain proteins can associate with specific acetylated histone residues, an interaction which can be disrupted by small molecules that competitively occupy the acetyl-lysing binding pockets in select members of this 61-member protein family (Filippakopoulos et al., 2012). Two recently developed bromodomain inhibitors, JQ1 and iBET, selectively bind to BRD4 (Filippakopoulos et al., 2010; Nicodeme et al., 2010). Despite this general role played in transcription regulation, inhibition of BRD4 by BET-inhibitors appears to have a highly selective effect on tumor cells (Dawson et al., 2011; Delmore et al., 2011; Mertz et al., 2011; Zuber et al., 2011). BET-inhibitors appear to cause dramatic suppression of the potent oncogene, MYC, and lead to a pronounced anti-proliferative effect in a range of tumors, including multiple myeloma (MM), Burkitt's lymphoma (BL), and acute myeloid leukemia (AML) (Dawson et al., 2011; Delmore et al., 2011; Mertz et al., 2011; Zuber et al., 2011). Although BRD4 inhibition shows great promise as a therapeutic agent in cancer, it remains unclear why inhibition of this general chromatin regulator has a selective effect on the MYC gene in these tumor cells.

To investigate this mechanism, we turned to concepts described Example 1 above. In that study, we demonstrated that transcriptional activators, such as the Mediator coactivator complex are not distributed evenly throughout the genome. Instead, we found that Mediator binding is concentrated at a discrete number of enhancer regions, which we have classified as super-enhancers. Our analysis of mouse embryonic stem cells (mESCs) revealed that these "super-enhancers" consist of enhancer clusters that span vast chromatin domains when compared to typical enhancer regions and are occupied by an order of magnitude more Mediator complex proteins. In addition, super-enhancers preferentially associate with and activate genes key to cell state.

Enhancers function through co-operative and synergistic interactions between multiple transcription factors and coactivators (Carey, 1998; Carey et al., 1990; Giese et al., 1995; Kim and Maniatis, 1997; Thanos and Maniatis, 1995). Cooperative binding and synergistic activation confer increased sensitivity, so that small changes in activator concentration can lead to dramatic changes in activator binding and transcription of associated genes (Carey, 1998). This led us to hypothesize that highly sensitive super-enhancers driving key oncogenic drivers in multiple myeloma may account for the selective effect of BRD4 inhibition.

In this study, we show that BRD4 inhibition has a highly selective effect on critical tumor genes associated with super-enhancers. As expected, given its role as a general regulator of transcriptional pause release and its association with the Mediator complex, we found that BRD4 was located at a majority of active enhancers and promoters in tumor cells. Strikingly, extreme levels of BRD4 were found at a small subset of enhancer regions, which we have termed super-enhancers. These regions are similar to the super-enhancers described in mouse embryonic stem cells as discussed in Example 1 above. We found that binding of BRD4 and Mediator at super-enhancers was hyper-sensitive to loss of BRD4 binding through BET inhibition. This in turn corresponded to a dramatic loss of transcription at super-enhancer associated genes, such as MYC. Our data suggest a model of how inhibitors of generally acting chromatin regulators can exert a gene-specific effect, through the disruption of heavily occupied, cooperatively bound sites functioning at highly expressed tumor regulators. This concept may improve our understanding of how these drugs should be selected for the treatment of genetically-defined cancers.

Results

Mediator and BRD4 Co-Occupy Promoters of Active Genes in Multiple Myeloma

In Example 1 above it was shown that Mediator and BRD4 co-occupy enhancers and active transcription start sites in embryonic stem cells and in differentiated cells. To determine whether Mediator and BRD4 co-occupy these sites in multiple myeloma cells, we used chromatin immunoprecipitation coupled to high-throughput sequencing (ChIP-Seq) with antibodies directed against Mediator, Brd4 and various marks of enhancers and active transcription start sites in MM.1S cells (FIG. **6**). The results, whether viewed by individual genes tracks or by meta-gene analysis, show that Mediator and BRD4 generally co-occupy enhancers and active transcription start sites (FIG. **6**A, B). Signals for Mediator and BRD4 were found together with those for nucleosomes with the histone modification H3K27Ac in 8,000 regions lacking transcription start sites, and these were considered enhancers. Signals for BRD4 and Mediator were also found together with those for the histone modification H3K4me3 and RNA polymerase II at 14,000 annotated transcription start sites, and these were considered active transcription start sites. The levels of Mediator and BRD4 occupancy correlated with one another at both enhancers and transcription start sites (FIG. **6**C), and the levels of BRD4 were correlated with the levels of RNA polymerase II at genes (FIG. **6**D), consistent with the results observed in non-tumor cells in Example 1 above. These results indicate that Mediator and BRD4 generally co-occupy enhancers and active transcription start sites throughout the genome of MM.1S cells.

Super-Enhancers are Associated with Key Multiple Myeloma Genes

The sizes of enhancers identified by Mediator occupancy showed an unusual distribution, with a small subset of enhancers containing exceptional levels of Mediator protein (FIG. **7**A). These 210 "super-enhancers" have features similar to those described in Example 1 above for mESCs (FIG. **7**A). These are regions occupied, on average, by 16-fold more Mediator compared to normal enhancer regions. Super-enhancers also occupy larger genomic regions than normal enhancers, with a median size of 20 kb, 16-fold greater than the normal enhancer size of 1.3 kb. In addition to high Mediator occupancy, these enhancers were also bound by exceptional levels of BRD4, on average, 16-fold higher than normal enhancers (FIG. **7**B).

As noted in Example 1 above, in ESCs and in differentiated cells, super-enhancers have exceptional transcription activation activity and are associated with highly expressed cell-type-specific genes that are located nearby. In MM.1S cells, super-enhancers were associated with highly expressed, cell-type specific genes, including genes known to be important in multiple myeloma (FIG. **7**C). For example, the MM.1S MYC locus contains a chromosomal rearrangement that places MYC under the control of the IgH enhancers, which are highly active in the antibody producing plasma cells from which MM derives. The IgH-MYC locus contains a large, 40 kb super-enhancer, occupied by high levels of both BRD4 and MED1 (FIG. **7**D). Super-enhancers were also found associated with the IRF4 gene (FIG. **7**D), which encodes a key plasma cell transcription factor frequently deregulated in MM (Shaffer et al., 2008).

BRD4 Occupancy at Super-Enhancers is Highly Sensitive to Bromodomain Inhibition

Enhancers are formed through co-operative and synergistic binding of multiple transcription factors and coactivators (Carey, 1998; Carey et al., 1990; Giese et al., 1995; Kim and Maniatis, 1997; Thanos and Maniatis, 1995). As a consequence of this binding behavior, enhancers bound by many cooperatively-interacting factors lose activity more rapidly than enhancers bound by fewer factors when the levels of enhancer-bound factors are reduced (Giniger and Ptashne, 1988; Griggs and Johnston, 1991). The presence of super-enhancers at MYC and other key genes associated with multiple myeloma led us to consider the hypothesis that super-enhancers are more sensitive to reduced levels of BRD4 than average enhancers. If super-enhancers are more sensitive to reduced levels of BRD4 than average enhancers, then super-enhancers should experience greater loss of BRD4 than average enhancers, and genes associated with super-enhancers might then experience a greater reduction of transcription than genes with average enhancers.

To test this hypothesis, we first examined the effects of various concentrations of JQ1 on genome-wide on BRD4 occupancy (FIG. **8**A). During the course of the 6 hour treatments, JQ1 had little effect on MM1.S cell viability, as measured by ATP levels, while at later time points, JQ1 had a dramatic antiproliferative effect (FIG. **8**B). As expected, MYC protein levels were significantly depleted by JQ1 treatment (FIG. **8**C)(Delmore et al., 2011). In contrast, JQ1 did not affect BRD4 protein levels within cells, and did not significantly reduce ChIP efficiency (FIG. **8**D), However, super-enhancers showed a greater loss of BRD4 occupancy when compared to regions with average or low amounts of BRD4 (FIG. **8**E). The IgH enhancer was among those super-enhancers that showed significantly greater loss of BRD4 than typical enhancer regions with lower BRD4 occupancy, such as CD28 (FIG. **8**G).

Loss of P-TEFb Accompanies BRD4 Inhibition

BRD4 recruits the active form of the positive transcription elongation factor P-TEFb, which stimulates pause release and transcription elongation (Bisgrove et al., 2007; Hargreaves et al., 2009; Jang et al., 2005; Jiang et al., 1998; Wu and Chiang, 2007; Wu et al., 2003; Yang et al., 2005). We used ChIP-Seq to investigate the global occupancy of P-TEFb in MM.1S cells and found that it generally occupies sites bound by Mediator and BRD4 (FIG. **9**A). We next investigated whether the loss of BRD4 observed with JQ1 treatment is accompanied by loss of P-TEFb at enhancers and transcription start sites. JQ1 treatment did indeed reduce the levels of P-TEFb at sites where there was a reduction in BRD4 (FIG. **9**B). Furthermore, P-TEFb was disproportionately lost at super-enhancers when compared to normal enhancers (FIG. **9**C). We

conclude that BET bromodomain inhibition of BRD4 leads to loss of P-TEFb at enhancers and transcription start sites, and that the inhibition has more profound effects at super-enhancers than at average enhancers.

To determine whether the loss of P-TEFb results in an elongation defect, we performed ChIP-seq of RNA Polymerase II (Pol II) after JQ1 treatment. We found that JQ1 treatment led to a global defect in transcriptional elongation, characterized by a loss of PolIII in the gene body and 3' transcription termination regions (FIG. **10**). Further inspection of gene tracks revealed that key super-enhancer associated genes, including MYC, showed a dramatic defect in elongation (FIG. **10**B). Globally, super-enhancer associated genes, had larger elongation defects in response to JQ1 than genes associated with normal enhancers (FIG. **10**C). These results are consistent with the interpretation that genes driven by super-enhancers show more dramatic transcriptional defects due to reduced pause release and elongation of their transcripts.

Discussion

At present, inhibitors of chromatin regulators are gaining increased interest as potential therapeutic agents for treating cancer. Many chromatin regulators are understood to play general roles in the control of transcription, yet to reach significant clinical efficacy, small molecule inhibitors must have a selective effect on tumor cells. Several recent studies have shown that inhibition of the bromodomain protein BRD4 can indeed have a highly specific effect, causing the down regulation of key tumor drivers in several cancer types. In multiple myeloma, acute myeloid leukemia, and Burkitt's lymphoma, treatment with BET inhibitors led to a dramatic loss of MYC expression (Dawson et al., 2011; Delmore et al., 2011; Mertz et al., 2011; Zuber et al., 2011). Understanding how inhibitors of generally acting chromatin regulators can exert a selective effect will vastly improve our understanding of how these drugs should be selected for the treatment of genetically-defined cancers.

We have gained insight into this concept through our study of super-enhancers. We have found that, across many cell types, key regulators of cell state are associated with large, 10-40 kb enhancer domains, characterized by disproportionately high levels of MED1 binding and, as we have profiled in multiple myeloma, BRD4. Although these super-enhancers make up only a small percentage of the total number of enhancer regions, they account for a large fraction of total MED1 and BRD4 binding across the genome. Most significantly, we have found that super-enhancers are more sensitive to perturbation than typical enhancer regions.

We found that inhibition of BRD4 led to the dramatic loss of BRD4 and CDK9 binding at super-enhancers. In multiple myeloma, super-enhancers were associated with key oncogenic drivers, such as MYC. Disruption of super-enhancers by BRD4 inhibition led to a dramatic loss of expression of these critical tumor genes, accompanied by a potent antiproliferative effect.

Our results demonstrate that super-enhancers occupied by BRD4 regulate critical oncogenic drivers multiple myeloma and show that BRD4 inhibition leads to preferential disruption of these super-enhancers. This insight into the mechanism by which Brd4 inhibition causes selective loss of oncogene expression in these highly malignant blood cancers may have implications for future drug development in oncology. Many oncogenes critical to tumor cell function are highly expressed and may therefore be driven by super-enhancers. If so, preferential disruption of super-enhancer function may be a general approach to selectively inhibiting the oncogenic drivers of many tumor cells.

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TABLE 1

Super-enhancers from ESC. Based on NCBI Build 37

REGION_ID	CHROM	START	STOP
INT_STITCHED_45	chr1	13049615	13094765
INT_STITCHED_88	chr1	34130107	34134640
INT_STITCHED_100	chr1	36070190	36074608
INT_STITCHED_101	chr1	36111164	36118698
INT_STITCHED_108	chr1	37039139	37045411
INT_STITCHED_230	chr1	72260528	72261272
INT_STITCHED_237	chr1	72839563	72858199
INT_STITCHED_282	chr1	84857219	84887132
INT_STITCHED_315	chr1	91766947	91773527
INT_STITCHED_368	chr1	120538712	120545414
INT_STITCHED_372	chr1	120971968	120973737
INT_STITCHED_374	chr1	121201424	121202481
INT_STITCHED_376	chr1	121295085	121296031
INT_STITCHED_449	chr1	137071028	137096284
INT_STITCHED_464	chr1	138586629	138593131
INT_STITCHED_466	chr1	138841643	138850970
INT_STITCHED_508	chr1	154939892	154943709
INT_STITCHED_556	chr1	168054897	168073079
INT_STITCHED_559	chr1	169201106	169220423
INT_STITCHED_610	chr1	182818684	182819554
INT_STITCHED_611	chr1	182854521	182864307
INT_STITCHED_615	chr1	183948212	183961841
INT_STITCHED_746	chr2	20574602	20591747
INT_STITCHED_803	chr2	30913257	30925299
INT_STITCHED_812	chr2	32008891	32030736
INT_STITCHED_817	chr2	33282029	33300860
INT_STITCHED_928	chr2	71488013	71494617
INT_STITCHED_931	chr2	71575856	71583914
INT_STITCHED_1196	chr2	152002668	152003777
INT_STITCHED_1198	chr2	152552277	152563676
INT_STITCHED_1210	chr2	154242651	154254374
INT_STITCHED_1256	chr2	162856904	162860933
INT_STITCHED_1257	chr2	162877048	162893236
INT_STITCHED_1279	chr2	165981373	165983444
INT_STITCHED_1300	chr2	168589688	168617170
INT_STITCHED_1392	chr3	9641461	9655131
INT_STITCHED_1480	chr3	34544904	34553511
INT_STITCHED_1482	chr3	34633687	34660705
INT_STITCHED_1607	chr3	88375442	88380083
INT_STITCHED_1626	chr3	95455034	95468269
INT_STITCHED_1629	chr3	96380383	96382115

TABLE 1-continued

Super-enhancers from ESC. Based on NCBI Build 37

REGION_ID	CHROM	START	STOP
INT_STITCHED_1630	chr3	96479158	96484864
INT_STITCHED_1658	chr3	103008304	103019058
INT_STITCHED_1732	chr3	129247012	129261362
INT_STITCHED_1744	chr3	133181431	133197648
INT_STITCHED_1749	chr3	135208956	135210744
INT_STITCHED_1973	chr4	55469259	55491081
INT_STITCHED_2076	chr4	98507649	98514709
INT_STITCHED_2152	chr4	118743867	118745786
INT_STITCHED_2175	chr4	123300547	123303179
INT_STITCHED_2192	chr4	125211671	125223450
INT_STITCHED_2205	chr4	126875757	126879027
INT_STITCHED_2223	chr4	130178808	130180168
INT_STITCHED_2224	chr4	130195646	130196547
INT_STITCHED_2265	chr4	137148873	137153839
INT_STITCHED_2268	chr4	137329436	137357766
INT_STITCHED_2273	chr4	138000554	138006368
INT_STITCHED_2291	chr4	140826072	140840922
INT_STITCHED_2292	chr4	141120768	141126477
INT_STITCHED_2295	chr4	141616653	141627603
INT_STITCHED_2297	chr4	141721916	141726166
INT_STITCHED_2317	chr4	147459254	147463850
INT_STITCHED_2354	chr4	154537213	154538078
INT_STITCHED_2355	chr4	154563584	154564383
INT_STITCHED_2465	chr5	33873714	33880481
INT_STITCHED_2510	chr5	53933177	53947327
INT_STITCHED_2535	chr5	65255735	65256794
INT_STITCHED_2712	chr5	113758941	113775389
INT_STITCHED_2736	chr5	116845764	116860853
INT_STITCHED_2745	chr5	118884660	118896412
INT_STITCHED_2746	chr5	118951444	118960269
INT_STITCHED_2752	chr5	120029649	120037063
INT_STITCHED_2754	chr5	120129592	120171482
INT_STITCHED_2770	chr5	123584659	123590728
INT_STITCHED_2830	chr5	135417523	135421698
INT_STITCHED_3005	chr6	31834643	31852445
INT_STITCHED_3044	chr6	39370384	39371286
INT_STITCHED_3045	chr6	39395571	39396779
INT_STITCHED_3120	chr6	64961359	64985161
INT_STITCHED_3130	chr6	67061148	67064202
INT_STITCHED_3184	chr6	83839914	83844315
INT_STITCHED_3217	chr6	91640161	91661247
INT_STITCHED_3342	chr6	122290093	122293017
INT_STITCHED_3347	chr6	122612514	122614260
INT_STITCHED_3348	chr6	122640118	122657871
INT_STITCHED_3349	chr6	122714316	122720862
INT_STITCHED_3360	chr6	125383335	125398024
INT_STITCHED_3429	chr6	142458188	142461905
INT_STITCHED_3437	chr6	143047309	143065758
INT_STITCHED_3450	chr6	145223385	145225674
INT_STITCHED_3467	chr7	3193004	3218183
INT_STITCHED_3475	chr7	4772296	4777612
INT_STITCHED_3481	chr7	13599334	13600325
INT_STITCHED_3523	chr7	30982397	30983339
INT_STITCHED_3525	chr7	31248315	31250619
INT_STITCHED_3550	chr7	38812914	38816123
INT_STITCHED_3568	chr7	52806853	52814768
INT_STITCHED_3576	chr7	56592909	56604632
INT_STITCHED_3601	chr7	71092246	71102481
INT_STITCHED_3652	chr7	86355826	86368339
INT_STITCHED_3658	chr7	87159908	87169963
INT_STITCHED_3661	chr7	87274999	87276022
INT_STITCHED_3662	chr7	87333420	87345334
INT_STITCHED_3685	chr7	91027196	91051830
INT_STITCHED_3765	chr7	119831735	119835688
INT_STITCHED_3856	chr7	140304156	140307245
INT_STITCHED_3890	chr7	147131117	147136231
INT_STITCHED_3914	chr7	152036872	152050716
INT_STITCHED_3947	chr8	12499468	12504771
INT_STITCHED_4014	chr8	35023426	35027483
INT_STITCHED_4033	chr8	37602064	37613850
INT_STITCHED_4034	chr8	37642521	37671979
INT_STITCHED_4046	chr8	44405736	44406755
INT_STITCHED_4116	chr8	74834685	74840663
INT_STITCHED_4163	chr8	87174072	87174643
INT_STITCHED_4167	chr8	87996475	87997654
INT_STITCHED_4179	chr8	91514813	91540176

TABLE 1-continued

Super-enhancers from ESC. Based on NCBI Build 37

REGION_ID	CHROM	START	STOP
INT_STITCHED_4190	chr8	93351924	93355292
INT_STITCHED_4546	chr9	56382386	56395769
INT_STITCHED_4555	chr9	58119837	58128504
INT_STITCHED_4657	chr9	78207143	78223442
INT_STITCHED_4748	chr9	110849422	110863371
INT_STITCHED_4766	chr9	114458126	114474355
INT_STITCHED_4797	chr9	120585871	120600072
INT_STITCHED_4802	chr9	121244501	121254102
INT_STITCHED_4885	chr10	20802131	20830236
INT_STITCHED_4891	chr10	21546502	21549691
INT_STITCHED_4893	chr10	21700576	21708946
INT_STITCHED_4954	chr10	39977900	39978752
INT_STITCHED_4981	chr10	44110139	44112766
INT_STITCHED_5021	chr10	59420365	59437537
INT_STITCHED_5044	chr10	62346394	62361563
INT_STITCHED_5054	chr10	66380351	66383761
INT_STITCHED_5059	chr10	66546199	66564235
INT_STITCHED_5091	chr10	75335464	75345568
INT_STITCHED_5092	chr10	75400370	75401358
INT_STITCHED_5100	chr10	76655655	76662360
INT_STITCHED_5111	chr10	79508474	79515168
INT_STITCHED_5140	chr10	85002060	85006553
INT_STITCHED_5325	chr11	8466451	8486876
INT_STITCHED_5331	chr11	9015537	9017663
INT_STITCHED_5340	chr11	12357626	12370205
INT_STITCHED_5427	chr11	33427175	33451476
INT_STITCHED_5484	chr11	52173182	52184686
INT_STITCHED_5499	chr11	54767341	54785832
INT_STITCHED_5533	chr11	62324296	62327251
INT_STITCHED_5553	chr11	66733372	66746990
INT_STITCHED_5555	chr11	66824791	66838230
INT_STITCHED_5565	chr11	69517060	69522803
INT_STITCHED_5597	chr11	77697704	77718786
INT_STITCHED_5666	chr11	88481360	88491812
INT_STITCHED_5711	chr11	97517673	97524159
INT_STITCHED_5719	chr11	98823511	98826466
INT_STITCHED_5741	chr11	102190649	102193692
INT_STITCHED_5752	chr11	104150171	104167544
INT_STITCHED_5768	chr11	107296669	107310982
INT_STITCHED_5819	chr11	116943025	116953583
INT_STITCHED_5831	chr11	117833701	117838253
INT_STITCHED_5875	chr12	12790432	12795881
INT_STITCHED_5876	chr12	12810177	12811020
INT_STITCHED_5880	chr12	12933791	12950936
INT_STITCHED_5995	chr12	55407498	55415046
INT_STITCHED_6000	chr12	56587347	56607146
INT_STITCHED_6004	chr12	57385208	57400114
INT_STITCHED_6112	chr12	87807046	87820319
INT_STITCHED_6113	chr12	87839385	87846192
INT_STITCHED_6118	chr12	88239069	88245155
INT_STITCHED_6151	chr12	103940487	103953004
INT_STITCHED_6186	chr12	111655417	111656705
INT_STITCHED_6187	chr12	111709296	111710794
INT_STITCHED_6188	chr12	111725920	111743677
INT_STITCHED_6460	chr13	64069823	64082322
INT_STITCHED_6544	chr13	96295094	96306119
INT_STITCHED_6557	chr13	98052562	98062842
INT_STITCHED_6559	chr13	98202400	98225162

TABLE 1-continued

Super-enhancers from ESC. Based on NCBI Build 37

REGION_ID	CHROM	START	STOP
INT_STITCHED_6615	chr13	110418702	110442750
INT_STITCHED_6709	chr14	22293688	22308989
INT_STITCHED_6789	chr14	49273113	49283200
INT_STITCHED_6815	chr14	55704349	55705463
INT_STITCHED_6859	chr14	64118817	64131901
INT_STITCHED_6864	chr14	65251303	65269514
INT_STITCHED_6887	chr14	71022659	71035930
INT_STITCHED_6904	chr14	76894682	76915946
INT_STITCHED_6906	chr14	77015215	77030315
INT_STITCHED_6957	chr14	99738540	99755307
INT_STITCHED_6981	chr14	106250319	106260753
INT_STITCHED_6982	chr14	106296486	106304433
INT_STITCHED_7104	chr15	25654102	25704265
INT_STITCHED_7202	chr15	61918415	61924748
INT_STITCHED_7248	chr15	77168852	77187251
INT_STITCHED_7285	chr15	88539016	88539831
INT_STITCHED_7317	chr15	97198605	97227633
INT_STITCHED_7318	chr15	97422878	97425328
INT_STITCHED_7343	chr15	103349226	103353500
INT_STITCHED_7359	chr16	8758173	8779472
INT_STITCHED_7434	chr16	23099373	23103471
INT_STITCHED_7452	chr16	29657509	29668114
INT_STITCHED_7597	chr16	84769173	84780686
INT_STITCHED_7680	chr17	10549089	10570838
INT_STITCHED_7728	chr17	26631721	26648689
INT_STITCHED_7747	chr17	29209618	29218426
INT_STITCHED_7752	chr17	29587776	29588942
INT_STITCHED_7767	chr17	31939569	31956756
INT_STITCHED_7784	chr17	35639211	35642435
INT_STITCHED_7792	chr17	37110202	37134996
INT_STITCHED_7794	chr17	37209046	37217726
INT_STITCHED_7812	chr17	45593477	45596503
INT_STITCHED_7822	chr17	47640414	47649043
INT_STITCHED_7876	chr17	66818723	66836409
INT_STITCHED_7884	chr17	71096763	71100905
INT_STITCHED_7886	chr17	71177302	71179956
INT_STITCHED_7887	chr17	71213804	71222433
INT_STITCHED_7888	chr17	71241991	71250610
INT_STITCHED_8114	chr18	35202713	35203454
INT_STITCHED_8124	chr18	36412873	36414154
INT_STITCHED_8136	chr18	38538325	38551037
INT_STITCHED_8139	chr18	38760823	38761958
INT_STITCHED_8140	chr18	38788269	38796942
INT_STITCHED_8148	chr18	40467587	40468140
INT_STITCHED_8209	chr18	61787544	61788400
INT_STITCHED_8260	chr18	75504155	75505202
INT_STITCHED_8261	chr18	75520332	75527277
INT_STITCHED_8264	chr18	75738693	75745073
INT_STITCHED_8324	chr19	5835881	5847014
INT_STITCHED_8378	chr19	21858770	21866770
INT_STITCHED_8385	chr19	23139991	23170189
INT_STITCHED_8386	chr19	23207455	23208806
INT_STITCHED_8399	chr19	25553498	25564092
INT_STITCHED_8519	chr19	53523440	53535319
INT_STITCHED_8554	chrX	7578969	7597907
INT_STITCHED_8629	chrX	50098631	50114110

TABLE 2

Multiple Myeloma Super-enhancers. Based on Gene Build hg 18

REGION_ID	CHROM	START	STOP
3_MM1S_MED1_DMSO_2_11472_lociStitched	chr22	21597907	21632017
12_MM1S_MED1_DMSO_2_12661_lociStitched	chr3	142561889	142658635
5_MM1S_MED1_DMSO_2_11467_lociStitched	chr22	21520124	21576243
3_MM1S_MED1_DMSO_2_15142_lociStitched	chr6	7822980	7864682
27_MM1S_MED1_DMSO_2_15896_lociStitched	chr6	108969554	109119470
10_MM1S_MED1_DMSO_2_883_lociStitched	chr1	117943520	118031299
13_MM1S_MED1_DMSO_2_9297_lociStitched	chr2	37383079	37478117
7_MM1S_MED1_DMSO_2_1421_lociStitched	chr1	201502736	201564474
6_MM1S_MED1_DMSO_2_10778_lociStitched	chr20	29712568	29775967

TABLE 2-continued

Multiple Myeloma Super-enhancers. Based on Gene Build hg 18

REGION_ID	CHROM	START	STOP
4_MM1S_MED1_DMSO_2_3066_lociStitched	chr11	64939923	64979931
12_MM1S_MED1_DMSO_2_10818_lociStitched	chr20	31862228	31936793
15_MM1S_MED1_DMSO_2_19349_lociStitched	chrX	130689710	130790383
6_MM1S_MED1_DMSO_2_15061_lociStitched	chr6	235131	282880
MM1S_MED1_DMSO_2_4011	chr12	51868026	51890008
13_MM1S_MED1_DMSO_2_6359_lociStitched	chr16	11662193	11750399
5_MM1S_MED1_DMSO_2_19070_lociStitched	chrX	48652795	48690448
9_MM1S_MED1_DMSO_2_13894_lociStitched	chr4	185522607	185586220
2_MM1S_MED1_DMSO_2_15298_lociStitched	chr6	26263259	26281958
4_MM1S_MED1_DMSO_2_2709_lociStitched	chr11	10280174	10301780
7_MM1S_MED1_DMSO_2_11528_lociStitched	chr22	27516134	27555928
5_MM1S_MED1_DMSO_2_7255_lociStitched	chr17	29712450	29745538
9_MM1S_MED1_DMSO_2_9712_lociStitched	chr2	98426920	98498831
10_MM1S_MED1_DMSO_2_5371_lociStitched	chr14	90884807	90955651
3_MM1S_MED1_DMSO_2_7984_lociStitched	chr18	9050438	9074417
8_MM1S_MED1_DMSO_2_16690_lociStitched	chr7	55566748	55610180
1_MM1S_MED1_DMSO_2_935_lociStitched	chr1	148122391	148127826
3_MM1S_MED1_DMSO_2_3735_lociStitched	chr12	12748016	12781726
4_MM1S_MED1_DMSO_2_2546_lociStitched	chr10	125812311	125857688
2_MM1S_MED1_DMSO_2_1862_lociStitched	chr10	11242759	11275331
3_MM1S_MED1_DMSO_2_929_lociStitched	chr1	147470833	147491868
MM1S_MED1_DMSO_2_15293	chr6	26161696	26165891
3_MM1S_MED1_DMSO_2_9167_lociStitched	chr2	20254183	20289776
1_MM1S_MED1_DMSO_2_15301_lociStitched	chr6	26303073	26309499
11_MM1S_MED1_DMSO_2_17447_lociStitched	chr8	27264787	27340169
3_MM1S_MED1_DMSO_2_178_lociStitched	chr1	17094196	17113973
13_MM1S_MED1_DMSO_2_17882_lociStitched	chr8	120985081	121017049
3_MM1S_MED1_DMSO_2_1025_lociStitched	chr1	153174936	153197206
1_MM1S_MED1_DMSO_2_13984_lociStitched	chr5	1364911	1374105
MM1S_MED1_DMSO_2_15361	chr6	27964884	27972054
3_MM1S_MED1_DMSO_2_3071_lociStitched	chr11	65020047	65035435
5_MM1S_MED1_DMSO_2_18418_lociStitched	chr9	92710817	92746187
3_MM1S_MED1_DMSO_2_13885_lociStitched	chr4	185421650	185447815
5_MM1S_MED1_DMSO_2_9691_lociStitched	chr2	96554603	96584612
10_MM1S_MED1_DMSO_2_15652_lociStitched	chr6	52501063	52557406
MM1S_MED1_DMSO_2_7572	chr17	53760011	53773039
6_MM1S_MED1_DMSO_2_15868_lociStitched	chr6	106637997	106665835
1_MM1S_MED1_DMSO_2_15308_lociStitched	chr6	26377785	26382951
2_MM1S_MED1_DMSO_2_7420_lociStitched	chr17	38792419	38802756
9_MM1S_MED1_DMSO_2_14628_lociStitched	chr5	131818986	131870127
3_MM1S_MED1_DMSO_2_13539_lociStitched	chr4	90429430	90459112
4_MM1S_MED1_DMSO_2_12859_lociStitched	chr3	178538717	178562722
4_MM1S_MED1_DMSO_2_4371_lociStitched	chr12	107533824	107560420
4_MM1S_MED1_DMSO_2_15314_lociStitched	chr6	26449533	26475951
MM1S_MED1_DMSO_2_15291	chr6	26138365	26142878
1_MM1S_MED1_DMSO_2_15296_lociStitched	chr6	26230241	26235063
9_MM1S_MED1_DMSO_2_5477_lociStitched	chr14	105096168	105120688
2_MM1S_MED1_DMSO_2_12120_lociStitched	chr3	46220865	46232443
MM1S_MED1_DMSO_2_1 5292	chr6	26150596	26154952
2_MM1S_MED1_DMSO_2_5546_lociStitched	chr15	29333964	29348240
1_MM1S_MED1_DMSO_2_176_lociStitched	chr1	16712194	16713944
1_MM1S_MED1_DMSO_2_12853_lociStitched	chr3	178395376	178403353
1_MM1S_MED1_DMSO_2_10897_lociStitched	chr20	36931952	36938862
2_MM1S_MED1_DMSO_2_9810_lociStitched	chr2	112172513	112182538
5_MM1S_MED1_DMSO_2_497_lociStitched	chr1	44945879	44970311
7_MM1S_MED1_DMSO_2_8152_lociStitched	chr18	44693277	44734029
1_MM1S_MED1_DMSO_2_3010_lociStitched	chr11	62362909	62367338
2_MM1S_MED1_DMSO_2_1718_lociStitched	chr1	232800286	232816291
7_MM1S_MED1_DMSO_2_16140_lociStitched	chr6	138287960	138339719
4_MM1S_MED1_DMSO_2_16924_lociStitched	chr7	101851129	101879762
7_MM1S_MED1_DMSO_2_3539_lociStitched	chr11	128090989	128134946
5_MM1S_MED1_DMSO_2_13905_lociStitched	chr4	185603808	185634087
5_MM1S_MED1_DMSO_2_8400_lociStitched	chr19	2546568	2579792
4_MM1S_MED1_DMSO_2_17232_lociStitched	chr7	149685067	149715545
9_MM1S_MED1_DMSO_2_6090_lociStitched	chr15	88364067	88447544
7_MM1S_MED1_DMSO_2_5551_lociStitched	chr15	29404247	29447806
6_MM1S_MED1_DMSO_2_908_lociStitched	chr1	144138338	144169442
6_MM1S_MED1_DMSO_2_2813_lociStitched	chr11	22633909	22661308
2_MM1S_MED1_DMSO_2_11309_lociStitched	chr21	40247390	40265606
3_MM1S_MED1_DMSO_2_11459_lociStitched	chr22	21406975	21431657
4_MM1S_MED1_DMSO_2_4023_lociStitched	chr12	52133823	52163301
3_MM1S_MED1_DMSO_2_6783_lociStitched	chr16	78185190	78197918
2_MM1S_MED1_DMSO_2_2451_lociStitched	chr10	112094075	112109393
4_MM1S_MED1_DMSO_2_3671_lociStitched	chr12	6916226	6942174
3_MM1S_MED1_DMSO_2_11367_lociStitched	chr21	44381407	44405755
6_MM1S_MED1_DMSO_2_18632_lociStitched	chr9	122670221	122707139

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REGION_ID	CHROM	START	STOP
5_MM1S_MED1_DMSO_2_7098_lociStitched	chr17	16810645	16836243
5_MM1S_MED1_DMSO_2_12822_lociStitched	chr3	173284485	173309559
3_MM1S_MED1_DMSO_2_7795_lociStitched	chr17	72647302	72672300
5_MM1S_MED1_DMSO_2_14194_lociStitched	chr5	55473448	55500561
4_MM1S_MED1_DMSO_2_5843_lociStitched	chr15	63374708	63385346
7_MM1S_MED1_DMSO_2_12921_lociStitched	chr3	184711984	184757118
3_MM1S_MED1_DMSO_2_13004_lociStitched	chr3	195330092	195342991
6_MM1S_MED1_DMSO_2_1869_lociStitched	chr10	11323723	11353214
6_MM1S_MED1_DMSO_2_5884_lociStitched	chr15	66355713	66386773
4_MM1S_MED1_DMSO_2_16493_lociStitched	chr7	25953531	25975640
2_MM1S_MED1_DMSO_2_17945_lociStitched	chr8	128815143	128831262
3_MM1S_MED1_DMSO_2_6443_lociStitched	chr16	23241697	23269855
1_MM1S_MED1_DMSO_2_15307_lociStitched	chr6	26356880	26361949
4_MM1S_MED1_DMSO_2_1007_lociStitched	chr1	152636911	152660538
12_MM1S_MED1_DMSO_2_12617_lociStitched	chr3	134643043	134708940
3_MM1S_MED1_DMSO_2_1629_lociStitched	chr1	224363473	224383373
3_MM1S_MED1_DMSO_2_2794_lociStitched	chr11	19406910	19422183
2_MM1S_MED1_DMSO_2_4947_lociStitched	chr13	113545919	113557086
3_MM1S_MED1_DMSO_2_15146_lociStitched	chr6	7903492	7922524
2_MM1S_MED1_DMSO_2_9355_lociStitched	chr2	43297983	43310825
MM1S_MED1_DMSO_2_15353	chr6	27882353	27887636
1_MM1S_MED1_DMSO_2_117_lociStitched	chr1	11889871	11893140
5_MM1S_MED1_DMSO_2_11097_lociStitched	chr20	55481270	55509295
1_MM1S_MED1_DMSO_2_10440_lociStitched	chr2	231437101	231447701
3_MM1S_MED1_DMSO_2_340_lociStitched	chr1	30988720	31005936
7_MM1S_MED1_DMSO_2_15801_lociStitched	chr6	90115755	90142733
3_MM1S_MED1_DMSO_2_9401_lociStitched	chr2	47380900	47404415
1_MM1S_MED1_DMSO_2_15359_lociStitched	chr6	27939690	27944056
6_MM1S_MED1_DMSO_2_16939_lociStitched	chr7	104350354	104392312
4_MM1S_MED1_DMSO_2_14621_lociStitched	chr5	131777514	131802069
9_MM1S_MED1_DMSO_2_7852_lociStitched	chr17	74224147	74290965
5_MM1S_MED1_DMSO_2_10765_lociStitched	chr20	25209731	25248761
3_MM1S_MED1_DMSO_2_11306_lociStitched	chr21	40217819	40231333
1_MM1S_MED1_DMSO_2_4955_lociStitched	chr13	113847326	113853279
9_MM1S_MED1_DMSO_2_17774_lociStitched	chr8	96022708	96074048
1_MM1S_MED1_DMSO_2_15467_lociStitched	chr6	33042969	33050991
2_MM1S_MED1_DMSO_2_10245_lociStitched	chr2	201688028	201701230
2_MM1S_MED1_DMSO_2_3620_lociStitched	chr12	4086510	4100254
1_MM1S_MED1_DMSO_2_11604_lociStitched	chr22	35056163	35061482
5_MM1S_MED1_DMSO_2_8117_lociStitched	chr18	40542132	40560323
5_MM1S_MED1_DMSO_2_17304_lociStitched	chr8	2016787	2037760
11_MM1S_MED1_DMSO_2_7624_lociStitched	chr17	59486930	59536700
5_MM1S_MED1_DMSO_2_7793_lociStitched	chr17	72590686	72618288
2_MM1S_MED1_DMSO_2_15176_lociStitched	chr6	11937666	11944210
3_MM1S_MED1_DMSO_2_8375_lociStitched	chr19	2032758	2049163
5_MM1S_MED1_DMSO_2_10377_lociStitched	chr2	219449340	219471887
1_MM1S_MED1_DMSO_2_18431_lociStitched	chr9	92992632	92996907
MM1S_MED1_DMSO_2_8809	chr19	44583388	44595931
6_MM1S_MED1_DMSO_2_19132_lociStitched	chrX	58141354	58176568
2_MM1S_MED1_DMSO_2_11329_lociStitched	chr21	42353240	42371485
3_MM1S_MED1_DMSO_2_3939_lociStitched	chr12	46487401	46506636
2_MM1S_MED1_DMSO_2_2457_lociStitched	chr10	112205500	112215498
6_MM1S_MED1_DMSO_2_6074_lociStitched	chr15	87434644	87475737
2_MM1S_MED1_DMSO_2_1061_lociStitched	chr1	154382144	154399688
6_MM1S_MED1_DMSO_2_14486_lociStitched	chr5	109279819	109314997
2_MM1S_MED1_DMSO_2_218_lociStitched	chr1	23723110	23739682
6_MM1S_MED1_DMSO_2_11882_lociStitched	chr3	5197581	5231167
5_MM1S_MED1_DMSO_2_8393_lociStitched	chr19	2419984	2446976
1_MM1S_MED1_DMSO_2_11487_lociStitched	chr22	22514623	22522474
5_MM1S_MED1_DMSO_2_11633_lociStitched	chr22	35940694	35972007
4_MM1S_MED1_DMSO_2_13300_lociStitched	chr4	39868398	39884094
2_MM1S_MED1_DMSO_2_15875_lociStitched	chr6	106717009	106735272
4_MM1S_MED1_DMSO_2_16685_lociStitched	chr7	55537132	55553461
2_MM1S_MED1_DMSO_2_13593_lociStitched	chr4	105626955	105636498
1_MM1S_MED1_DMSO_2_5492_lociStitched	chr14	105394828	105400642
2_MM1S_MED1_DMSO_2_1032_lociStitched	chr1	153236845	153257390
6_MM1S_MED1_DMSO_2_6769_lociStitched	chr16	77326423	77362760
4_MM1S_MED1_DMSO_2_15040_lociStitched	chr5	180161278	180192831
2_MM1S_MED1_DMSO_2_11510_lociStitched	chr22	25335621	25345570
4_MM1S_MED1_DMSO_2_5303_lociStitched	chr14	76557983	76580142
3_MM1S_MED1_DMSO_2_15065_lociStitched	chr6	334189	345497
2_MM1S_MED1_DMSO_2_10912_lociStitched	chr20	40143996	40158547
5_MM1S_MED1_DMSO_2_6691_lociStitched	chr16	66841952	66878349
1_MM1S_MED1_DMSO_2_7334_lociStitched	chr17	35163138	35168797
2_MM1S_MED1_DMSO_2_18434_lociStitched	chr9	93221024	93234776
3_MM1S_MED1_DMSO_2_8242_lociStitched	chr18	58955785	58981327

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REGION_ID	CHROM	START	STOP
1_MM1S_MED1_DMSO_2_13003_lociStitched	chr3	195300012	195305617
2_MM1S_MED1_DMSO_2_6646_lociStitched	chr16	65106878	65117734
3_MM1S_MED1_DMSO_2_4266_lociStitched	chr12	93065052	93093164
3_MM1S_MED1_DMSO_2_11259_lociStitched	chr21	35158227	35184979
4_MM1S_MED1_DMSO_2_3801_lociStitched	chr12	26157584	26171339
2_MM1S_MED1_DMSO_2_16133_lociStitched	chr6	138228659	138247051
3_MM1S_MED1_DMSO_2_17236_lociStitched	chr7	149731864	149749863
4_MM1S_MED1_DMSO_2_13002_lociStitched	chr3	195258091	195287025
2_MM1S_MED1_DMSO_2_953_lociStitched	chr1	148798802	148808298
2_MM1S_MED1_DMSO_2_1450_lociStitched	chr1	203508812	203524935
2_MM1S_MED1_DMSO_2_15283_lociStitched	chr6	25511304	25522342
3_MM1S_MED1_DMSO_2_290_lociStitched	chr1	26890818	26902191
5_MM1S_MED1_DMSO_2_7990_lociStitched	chr18	9091649	9111559
7_MM1S_MED1_DMSO_2_18762_lociStitched	chr9	133102585	133143969
1_MM1S_MED1_DMSO_2_11360_lociStitched	chr21	44021842	44029128
2_MM1S_MED1_DMSO_2_3442_lociStitched	chr11	118244109	118249498
3_MM1S_MED1_DMSO_2_240_lociStitched	chr1	24384810	24406266
3_MM1S_MED1_DMSO_2_13402_lociStitched	chr4	71744317	71766940
1_MM1S_MED1_DMSO_2_1504_lociStitched	chr1	207342554	207349164
2_MM1S_MED1_DMSO_2_3411_lociStitched	chr11	114631374	114641681
1_MM1S_MED1_DMSO_2_6445_lociStitched	chr16	23321100	23326979
3_MM1S_MED1_DMSO_2_15550_lociStitched	chr6	37230628	37252404
2_MM1S_MED1_DMSO_2_13986_lociStitched	chr5	1388551	1399215
4_MM1S_MED1_DMSO_2_1441_lociStitched	chr1	202729083	202757890
4_MM1S_MED1_DMSO_2_1469_lociStitched	chr1	204784341	204809621
1_MM1S_MED1_DMSO_2_10460_lociStitched	chr2	232278796	232285774
2_MM1S_MED1_DMSO_2_2970_lociStitched	chr11	60354930	60369771
3_MM1S_MED1_DMSO_2_8650_lociStitched	chr19	16555465	16572388
9_MM1S_MED1_DMSO_2_12125_lociStitched	chr3	46292850	46331709
1_MM1S_MED1_DMSO_2_16944_lociStitched	chr7	104438848	104443908
3_MM1S_MED1_DMSO_2_19007_lociStitched	chrX	39838174	39854463
1_MM1S_MED1_DMSO_2_3626_lociStitched	chr12	4247853	4257225
2_MM1S_MED1_DMSO_2_14483_lociStitched	chr5	109219736	109229823
6_MM1S_MED1_DMSO_2_12115_lociStitched	chr3	46081401	46126461
3_MM1S_MED1_DMSO_2_11151_lociStitched	chr20	61828935	61842486
4_MM1S_MED1_DMSO_2_5613_lociStitched	chr15	38175241	38196125
4_MM1S_MED1_DMSO_2_13278_lociStitched	chr4	37983729	37998765
6_MM1S_MED1_DMSO_2_5325_lociStitched	chr14	81000404	81025576
5_MM1S_MED1_DMSO_2_8632_lociStitched	chr19	16112417	16131135
7_MM1S_MED1_DMSO_2_6134_lociStitched	chr15	91147531	91189935
1_MM1S_MED1_DMSO_2_7450_lociStitched	chr17	40653952	40663191
5_MM1S_MED1_DMSO_2_1463_lociStitched	chr1	204455280	204477658
3_MM1S_MED1_DMSO_2_12583_lociStitched	chr3	130511014	130530874
1_MM1S_MED1_DMSO_2_19115_lociStitched	chrX	56805175	56811038
2_MM1S_MED1_DMSO_2_811_lociStitched	chr1	110963171	110982799
1_MM1S_MED1_DMSO_2_7885_lociStitched	chr17	77090061	77097539
3_MM1S_MED1_DMSO_2_11917_lociStitched	chr3	13010123	13036559
5_MM1S_MED1_DMSO_2_2179_lociStitched	chr10	73677336	73694126
3_MM1S_MED1_DMSO_2_8045_lociStitched	chr18	19057373	19077707
1_MM1S_MED1_DMSO_2_14417_lociStitched	chr5	90711139	90716188
2_MM1S_MED1_DMSO_2_4222_lociStitched	chr12	88262387	88273597
1_MM1S_MED1_DMSO_2_1055_lociStitched	chr1	154210608	154218896
4_MM1S_MED1_DMSO_2_13888_lociStitched	chr4	185476602	185507051
5_MM1S_MED1_DMSO_2_13340_lociStitched	chr4	47873764	47901113
3_MM1S_MED1_DMSO_2_902_lociStitched	chr1	144093230	144111474
4_MM1S_MED1_DMSO_2_4375_lociStitched	chr12	107581795	107622903
3_MM1S_MED1_DMSO_2_7801_lociStitched	chr17	72740997	72755489
2_MM1S_MED1_DMSO_2_2458_lociStitched	chr10	112245714	112254934
11_MM1S_MED1_DMSO_2_14962_lociStitched	chr5	173243900	173289403
4_MM1S_MED1_DMSO_2_12387_lociStitched	chr3	99962343	99978843
1_MM1S_MED1_DMSO_2_4479_lociStitched	chr12	119212631	119215958
3_MM1S_MED1_DMSO_2_15872_lociStitched	chr6	106692441	106702198
2_MM1S_MED1_DMSO_2_4368_lociStitched	chr12	107478295	107494548
3_MM1S_MED1_DMSO_2_8359_lociStitched	chr19	1598817	1620929
5_MM1S_MED1_DMSO_2_11087_lociStitched	chr20	55390112	55408865
5_MM1S_MED1_DMSO_2_3367_lociStitched	chr11	110737473	110765459
1_MM1S_MED1_DMSO_2_17494_lociStitched	chr8	29685550	29690431
5_MM1S_MED1_DMSO_2_9346_lociStitched	chr2	42179512	42210718
9_MM1S_MED1_DMSO_2_12137_lociStitched	chr3	46384095	46413568
4_MM1S_MED1_DMSO_2_2241_lociStitched	chr10	80670951	80690429
3_MM1S_MED1_DMSO_2_18151_lociStitched	chr9	9596419	9605712
4_MM1S_MED1_DMSO_2_5951_lociStitched	chr15	72850107	72865537
6_MM1S_MED1_DMSO_2_9773_lociStitched	chr2	109176497	109219823
2_MM1S_MED1_DMSO_2_11509_lociStitched	chr22	25312119	25321438
2_MM1S_MED1_DMSO_2_13009_lociStitched	chr3	195504620	195516950
4_MM1S_MED1_DMSO_2_11609_lociStitched	chr22	35102731	35115007

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REGION_ID	CHROM	START	STOP
7_MM1S_MED1_DMSO_2_5487_lociStitched	chr14	105217337	105240489
1_MM1S_MED1_DMSO_2_10174_lociStitched	chr2	192248312	192253669
3_MM1S_MED1_DMSO_2_7356_lociStitched	chr17	35720293	35737137
4_MM1S_MED1_DMSO_2_1849_lociStitched	chr10	7553027	7575263
4_MM1S_MED1_DMSO_2_16642_lociStitched	chr7	47479754	47504874
3_MM1S_MED1_DMSO_2_14100_lociStitched	chr5	32607763	32625969
5_MM1S_MED1_DMSO_2_4572_lociStitched	chr12	123957232	123991926
4_MM1S_MED1_DMSO_2_2466_lociStitched	chr10	112590984	112617972
1_MM1S_MED1_DMSO_2_11372_lociStitched	chr21	44484038	44489285
8_MM1S_MED1_DMSO_2_8848_lociStitched	chr19	46720121	46762201
2_MM1S_MED1_DMSO_2_2455_lociStitched	chr10	112162427	112174574
3_MM1S_MED1_DMSO_2_6671_lociStitched	chr16	66137623	66158813
3_MM1S_MED1_DMSO_2_19339_lociStitched	chrX	130662431	130673564
2_MM1S_MED1_DMSO_2_1640_lociStitched	chr1	224906864	224919127
3_MM1S_MED1_DMSO_2_7431_lociStitched	chr17	39630911	39655723
1_MM1S_MED1_DMSO_2_8114_lociStitched	chr18	40512420	40517489
1_MM1S_MED1_DMSO_2_14692_lociStitched	chr5	138749622	138758419
3_MM1S_MED1_DMSO_2_1123_lociStitched	chr1	158943494	158980488
4_MM1S_MED1_DMSO_2_16250_lociStitched	chr6	157897104	157913718
2_MM1S_MED1_DMSO_2_17101_lociStitched	chr7	130440752	130460529
3_MM1S_MED1_DMSO_2_9135_lociStitched	chr2	11801032	11812720
3_MM1S_MED1_DMSO_2_10929_lociStitched	chr20	42002450	42018666
3_MM1S_MED1_DMSO_2_1119_lociStitched	chr1	158908226	158921742
1_MM1S_MED1_DMSO_2_3090_lociStitched	chr11	65380453	65385752
2_MM1S_MED1_DMSO_2_16369_lociStitched	chr7	5531468	5539806
5_MM1S_MED1_DMSO_2_13495_lociStitched	chr4	84352012	84381789
2_MM1S_MED1_DMSO_2_17959_lociStitched	chr8	129734648	129741973
1_MM1S_MED1_DMSO_2_5907_lociStitched	chr15	68174162	68181768
4_MM1S_MED1_DMSO_2_10987_lociStitched	chr20	45820090	45849423
2_MM1S_MED1_DMSO_2_5964_lociStitched	chr15	73121792	73127799
2_MM1S_MED1_DMSO_2_17479_lociStitched	chr8	29253249	29266444
5_MM1S_MED1_DMSO_2_18891_lociStitched	chrX	10025533	10050677
2_MM1S_MED1_DMSO_2_17880_lociStitched	chr8	120954422	120969636
1_MM1S_MED1_DMSO_2_1813_lociStitched	chr10	3814293	3818876
5_MM1S_MED1_DMSO_2_9497_lociStitched	chr2	64716540	64748251
4_MM1S_MED1_DMSO_2_7748_lociStitched	chr17	71366025	71387309
6_MM1S_MED1_DMSO_2_2420_lociStitched	chr10	105235066	105265831
3_MM1S_MED1_DMSO_2_7698_lociStitched	chr17	68094083	68113162
2_MM1S_MED1_DMSO_2_18473_lociStitched	chr9	97295762	97314118
1_MM1S_MED1_DMSO_2_7313_lociStitched	chr17	34110323	34116969
7_MM1S_MED1_DMSO_2_4966_lociStitched	chr13	114042783	114062417
3_MM1S_MED1_DMSO_2_14016_lociStitched	chr5	6528481	6550072
1_MM1S_MED1_DMSO_2_10942_lociStitched	chr20	42704197	42716062
4_MM1S_MED1_DMSO_2_13987_lociStitched	chr5	1541550	1578016
3_MM1S_MED1_DMSO_2_13707_lociStitched	chr4	129949673	129960374
2_MM1S_MED1_DMSO_2_10549_lociStitched	chr2	238264326	238277907
4_MM1S_MED1_DMSO_2_19321_lociStitched	chrX	128720479	128739812
3_MM1S_MED1_DMSO_2_9743_lociStitched	chr2	105694868	105718268
1_MM1S_MED1_DMSO_2_9393_lociStitched	chr2	47061614	47068522
3_MM1S_MED1_DMSO_2_10581_lociStitched	chr2	241152963	241176172
5_MM1S_MED1_DMSO_2_2158_lociStitched	chr10	71906942	71940173
3_MM1S_MED1_DMSO_2_3127_lociStitched	chr11	66789831	66814109
2_MM1S_MED1_DMSO_2_12973_lociStitched	chr3	188262377	188274985
1_MM1S_MED1_DMSO_2_14147_lociStitched	chr5	43072552	43079610
5_MM1S_MED1_DMSO_2_7714_lociStitched	chr17	70249246	70279117
2_MM1S_MED1_DMSO_2_13272_lociStitched	chr4	37805684	37812390
1_MM1S_MED1_DMSO_2_9642_lociStitched	chr2	86073843	86082122
1_MM1S_MED1_DMSO_2_13666_lociStitched	chr4	121888607	121891728
2_MM1S_MED1_DMSO_2_6598_lociStitched	chr16	55501343	55510262
4_MM1S_MED1_DMSO_2_12038_lociStitched	chr3	39222524	39251963
2_MM1S_MED1_DMSO_2_1926_lociStitched	chr10	15866384	15871377
2_MM1S_MED1_DMSO_2_5835_lociStitched	chr15	62961116	62976322
2_MM1S_MED1_DMSO_2_11661_lociStitched	chr22	37031196	37044926
1_MM1S_MED1_DMSO_2_2098_lociStitched	chr10	63326304	63335210
4_MM1S_MED1_DMSO_2_3547_lociStitched	chr11	128727439	128752307
3_MM1S_MED1_DMSO_2_12969_lociStitched	chr3	188185946	188202148
2_MM1S_MED1_DMSO_2_11345_lociStitched	chr21	43454397	43471457
3_MM1S_MED1_DMSO_2_19091_lociStitched	chrX	52966103	52981642
3_MM1S_MED1_DMSO_2_8625_lociStitched	chr19	16041917	16058919
3_MM1S_MED1_DMSO_2_1534_lociStitched	chr1	209753487	209768728
3_MM1S_MED1_DMSO_2_2769_lociStitched	chr11	16923726	16943228

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REGION_ID	CHROM	START	STOP
18_U87_MED1_20020_lociStitched	chr3	45100470	45243521
12_U87_MED1_7111_lociStitched	chr12	64271490	64380497
22_U87_MED1_17388_lociStitched	chr2	237744314	237896194
8_U87_MED1_7790_lociStitched	chr12	126279637	126344656
16_U87_MED1_25966_lociStitched	chr6	44066339	44153887
20_U87_MED1_17421_lociStitched	chr2	237957090	238086756
13_U87_MED1_24508_lociStitched	chr5	135356769	135440815
5_U87_MED1_21695_lociStitched	chr3	195773224	195801953
10_U87_MED1_28029_lociStitched	chr7	100523787	100571097
6_U87_MED1_32110_lociStitched	chrX	45479800	45553892
U87_MED1_6148	chr11	121548066	121570391
19_U87_MED1_15336_lociStitched	chr2	46879598	46970410
6_U87_MED1_28324_lociStitched	chr7	130215723	130260073
17_U87_MED1_28910_lociStitched	chr8	23203324	23280028
6_U87_MED1_18087_lociStitched	chr20	45376522	45424087
4_U87_MED1_5653_lociStitched	chr11	64940094	64979948
9_U87_MED1_11378_lociStitched	chr16	76138395	76189426
2_U87_MED1_19517_lociStitched	chr3	4992550	5013365
9_U87_MED1_24067_lociStitched	chr5	90604451	90646666
6_U87_MED1_24844_lociStitched	chr5	150121686	150155852
5_U87_MED1_27721_lociStitched	chr7	72748931	72774831
10_U87_MED1_20211_lociStitched	chr3	55151891	55214347
6_U87_MED1_5659_lociStitched	chr11	64995165	65033129
3_U87_MED1_19044_lociStitched	chr22	28920868	28939971
8_U87_MED1_24834_lociStitched	chr5	149974547	150020460
13_U87_MED1_21089_lociStitched	chr3	142532100	142623859
19_U87_MED1_18679_lociStitched	chr21	38534163	38647146
11_U87_MED1_28206_lociStitched	chr7	115938214	116016989
3_U87_MED1_12623_lociStitched	chr17	55257387	55278945
15_U87_MED1_7069_lociStitched	chr12	61372699	61474955
U87_MED1_5680	chr11	65411528	65428724
U87_MED1_5682	chr11	65433153	65444824
U87_MED1_19439	chr22	44836466	44869626
2_U87_MED1_3956_lociStitched	chr10	73689550	73708761
4_U87_MED1_27840_lociStitched	chr7	81067427	81109206
3_U87_MED1_32102_lociStitched	chrX	45440741	45464841
5_U87_MED1_28952_lociStitched	chr8	24104754	24143595
21_U87_MED1_5003_lociStitched	chr11	12100995	12218222
11_U87_MED1_26945_lociStitched	chr6	158359374	158413800
10_U87_MED1_3959_lociStitched	chr10	73725225	73767483
5_U87_MED1_3340_lociStitched	chr10	17280959	17321940
5_U87_MED1_11362_lociStitched	chr16	75864680	75900842
4_U87_MED1_26256_lociStitched	chr6	86210398	86250125
10_U87_MED1_8940_lociStitched	chr14	61060615	61131816
7_U87_MED1_20945_lociStitched	chr3	128935531	128978089
7_U87_MED1_9371_lociStitched	chr14	95781765	95823179
7_U87_MED1_17803_lociStitched	chr20	29638882	29664742
6_U87_MED1_11435_lociStitched	chr16	81214233	81249274
8_U87_MED1_23347_lociStitched	chr5	14195028	14261996
21_U87_MED1_25200_lociStitched	chr5	172209298	172316383
6_U87_MED1_22301_lociStitched	chr4	74786901	74829347
8_U87_MED1_31350_lociStitched	chr9	117391466	117456436
2_U87_MED1_11091_lociStitched	chr16	55196851	55207509
3_U87_MED1_16900_lociStitched	chr2	207810793	207833238
14_U87_MED1_4025_lociStitched	chr10	76895156	76977932
7_U87_MED1_7635_lociStitched	chr12	119145594	119188677
15_U87_MED1_10048_lociStitched	chr15	65153353	65230563
12_U87_MED1_27257_lociStitched	chr7	22565898	22624022
1_U87_MED1_25943_lociStitched	chr6	43843596	43867854
4_U87_MED1_5758_lociStitched	chr11	68819807	68846515
3_U87_MED1_22330_lociStitched	chr4	75290119	75317605
12_U87_MED1_9569_lociStitched	chr15	30898025	30959213
14_U87_MED1_25174_lociStitched	chr5	172116992	172191454
15_U87_MED1_29037_lociStitched	chr8	28260823	28333470
5_U87_MED1_17455_lociStitched	chr2	238996356	239014679
4_U87_MED1_22339_lociStitched	chr4	75448510	75480580
2_U87_MED1_12177_lociStitched	chr17	35423480	35437302
9_U87_MED1_19052_lociStitched	chr22	28954187	29006184
5_U87_MED1_20569_lociStitched	chr3	100162550	100188309
9_U87_MED1_23186_lociStitched	chr4	189557679	189609237
11_U87_MED1_31536_lociStitched	chr9	129340908	129389008
4_U87_MED1_23355_lociStitched	chr5	14450202	14474301
U87_MED1_6146	chr11	121515959	121540976
8_U87_MED1_25729_lociStitched	chr6	30816520	30858966
10_U87_MED1_4983_lociStitched	chr11	12020084	12069159
16_U87_MED1_18248_lociStitched	chr20	51915427	52011299

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REGION_ID	CHROM	START	STOP
2_U87_MED1_23778_lociStitched	chr5	64520345	64541781
9_U87_MED1_7528_lociStitched	chr12	110318731	110366261
1_U87_MED1_7124_lociStitched	chr12	64501251	64520825
12_U87_MED1_12963_lociStitched	chr17	73791501	73869039
11_U87_MED1_19190_lociStitched	chr22	35053881	35115041
10_U87_MED1_21395_lociStitched	chr3	171889621	171955016
8_U87_MED1_1494_lociStitched	chr1	94946918	94980513
11_U87_MED1_18744_lociStitched	chr21	41905622	41959032
5_U87_MED1_16409_lociStitched	chr2	160767297	160807533
7_U87_MED1_24782_lociStitched	chr5	149368846	149428980
11_U87_MED1_30075_lociStitched	chr8	128970951	129032504
7_U87_MED1_3670_lociStitched	chr10	49466581	49513686
11_U87_MED1_96_lociStitched	chr1	7976379	8045080
7_U87_MED1_18338_lociStitched	chr20	58219251	58265651
7_U87_MED1_24799_lociStitched	chr5	149623193	149663216
13_U87_MED1_31238_lociStitched	chr9	113811355	113877599
11_U87_MED1_4127_lociStitched	chr10	80528198	80590428
2_U87_MED1_26448_lociStitched	chr6	112461732	112477579
7_U87_MED1_28103_lociStitched	chr7	105771944	105806491
18_U87_MED1_31033_lociStitched	chr9	100772868	100889180
14_U87_MED1_30675_lociStitched	chr9	37974257	38060126
8_U87_MED1_10549_lociStitched	chr15	99038601	99089992
11_U87_MED1_9182_lociStitched	chr14	76439874	76498969
7_U87_MED1_24848_lociStitched	chr5	150413621	150465246
6_U87_MED1_23363_lociStitched	chr5	14492548	14555243
3_U87_MED1_13121_lociStitched	chr18	3575542	3597033
12_U87_MED1_24585_lociStitched	chr5	138995233	139071634
6_U87_MED1_5284_1ociStitched	chr11	35116090	35159659
8_U87_MED1_3521_lociStitched	chr10	33284148	33345221
7_U87_MED1_3616_lociStitched	chr10	43658240	43708520
13_U87_MED1_9361_lociStitched	chr14	95621243	95685962
4_U87_MED1_1127_lociStitched	chr1	58992223	59025064
7_U87_MED1_12194_lociStitched	chr17	35693162	35742563
8_U87_MED1_16879_lociStitched	chr2	206252656	206308088
9_U87_MED1_8736_lociStitched	chr14	34901740	34946481
4_U87_MED1_31655_lociStitched	chr9	132701910	132727822
9_U87_MED1_28850_lociStitched	chr8	22269372	22319900
6_U87_MED1_1768_lociStitched	chr1	143784496	143840429
4_U87_MED1_103_lociStitched	chr1	8059282	8081510
7_U87_MED1_2854_lociStitched	chr1	222980231	223015835
8_U87_MED1_10117_lociStitched	chr15	68542055	68588784
7_U87_MED1_9901_lociStitched	chr15	58441168	58488832
3_U87_MED1_15883_lociStitched	chr2	101938979	101958391
3_U87_MED1_2303_lociStitched	chr1	181502080	181517873
3_U87_MED1_9993_lociStitched	chr15	63374614	63385055
5_U87_MED1_4927_lociStitched	chr11	10276396	10310109
8_U87_MED1_152_lociStitched	chr1	9145323	9194908
7_U87_MED1_9271_lociStitched	chr14	90758863	90795031
5_U87_MED1_292_lociStitched	chr1	16147097	16166359
9_U87_MED1_21428_lociStitched	chr3	173326232	173383239
3_U87_MED1_1480_lociStitched	chr1	94764000	94783945
8_U87_MED1_23764_lociStitched	chr5	64362972	64408615
3_U87_MED1_29946_lociStitched	chr8	123392549	123407278
7_U87_MED1_12906_lociStitched	chr17	72195710	72225546
8_U87_MED1_15990_lociStitched	chr2	113267162	113314982
7_U87_MED1_116_lociStitched	chr1	8176575	8201970
5_U87_MED1_3649_lociStitched	chr10	44783418	44814443
U87_MED1_12057	chr17	26944014	26950786
2_U87_MED1_28437_lociStitched	chr7	137207368	137218994
5_U87_MED1_12467_lociStitched	chr17	45458793	45498200
6_U87_MED1_7769_lociStitched	chr12	123954906	123991790
8_U87_MED1_31230_lociStitched	chr9	113739183	113777283
5_U87_MED1_31605_lociStitched	chr9	131283335	131311945
7_U87_MED1_13271_lociStitched	chr18	18500819	18546785
6_U87_MED1_11526_lociStitched	chr16	85154494	85187872
6_U87_MED1_10162_lociStitched	chr15	72000419	72046025
2_U87_MED1_23340_lociStitched	chr5	14085673	14095662
2_U87_MED1_19978_lociStitched	chr3	43871898	43892096
3_U87_MED1_6430_lociStitched	chr12	6511065	6534804
9_U87_MED1_19998_lociStitched	chr3	45053955	45084099
7_U87_MED1_16195_lociStitched	chr2	134284761	134318932
11_U87_MED1_23940_lociStitched	chr5	77835851	77885336
6_U87_MED1_27845_lociStitched	chr7	81142953	81184064
4_U87_MED1_17882_lociStitched	chr20	33353373	33372955
10_U87_MED1_28600_lociStitched	chr7	154610962	154658753
5_U87_MED1_4664_lociStitched	chr10	124030434	124058691

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REGION_ID	CHROM	START	STOP
4_U87_MED1_31381_lociStitched	chr9	118068405	118091501
3_U87_MED1_29735_lociStitched	chr8	103869131	103893137
5_U87_MED1_10189_lociStitched	chr15	72475467	72510666
10_U87_MED1_24811_lociStitched	chr5	149818464	149877985
7_U87_MED1_30097_lociStitched	chr8	129248470	129279733
3_U87_MED1_9036_lociStitched	chr14	68314932	68333600
5_U87_MED1_20051_lociStitched	chr3	46104252	46131876
4_U87_MED1_1138_lociStitched	chr1	59085582	59122632
4_U87_MED1_15701_lociStitched	chr2	75667467	75701638
4_U87_MED1_711_lociStitched	chr1	33565656	33589393
8_U87_MED1_13446_lociStitched	chr18	42508896	42556266
10_U87_MED1_30394_lociStitched	chr9	3846346	3907818
3_U87_MED1_19433_lociStitched	chr22	44770633	44789144
8_U87_MED1_12920_lociStitched	chr17	72792423	72841736
3_U87_MED1_29401_lociStitched	chr8	62831256	62843484
8_U87_MED1_23950_lociStitched	chr5	77897945	77947772
4_U87_MED1_11532_lociStitched	chr16	85244100	85274282
3_U87_MED1_17802_lociStitched	chr20	29610545	29626036
9_U87_MED1_7003_lociStitched	chr12	55806899	55852250
9_U87_MED1_30667_lociStitched	chr9	37919181	37959597
5_U87_MED1_23475_lociStitched	chr5	34599305	34646619
9_U87_MED1_21809_lociStitched	chr4	5774565	5812219
5_U87_MED1_2484_lociStitched	chr1	199931838	199958314
2_U87_MED1_18366_lociStitched	chr20	60595254	60615120
3_U87_MED1_7103_lociStitched	chr12	64204208	64218785
2_U87_MED1_20034_lociStitched	chr3	45560540	45571271
3_U87_MED1_12304_lociStitched	chr17	38789690	38802753
5_U87_MED1_22699_lociStitched	chr4	123904338	123939922
4_U87_MED1_9736_lociStitched	chr15	43521877	43538803
7_U87_MED1_11894_lociStitched	chr17	16864733	16908403
5_U87_MED1_22347_lociStitched	chr4	75606560	75632402
7_U87_MED1_3329_lociStitched	chr10	17067573	17112416
7_U87_MED1_11080_lociStitched	chr16	54061119	54092821
6_U87_MED1_3934_lociStitched	chr10	73013845	73035645
8_U87_MED1_9304_lociStitched	chr14	92160646	92214011
8_U87_MED1_131_lociStitched	chr1	8851431	8891614
6_U87_MED1_2968_lociStitched	chr1	232801400	232834869
7_U87_MED1_4111_lociStitched	chr10	80355085	80408481
7_U87_MED1_6339_lociStitched	chr12	2222492	2249299
4_U87_MED1_26068_lociStitched	chr6	52475692	52496081
10_U87_MED1_27934_lociStitched	chr7	93489372	93537292
5_U87_MED1_3808_lociStitched	chr10	64315142	64346977
3_U87_MED1_944_lociStitched	chr1	44945138	44970174
3_U87_MED1_18034_lociStitched	chr20	43147144	43169721
3_U87_MED1_325_lociStitched	chr1	17094881	17113779
3_U87_MED1_3042_lociStitched	chr1	238461272	238489689
9_U87_MED1_1156_lociStitched	chr1	59361216	59425669
6_U87_MED1_30197_lociStitched	chr8	134210870	134248321
3_U87_MED1_32076_lociStitched	chrX	43702439	43721105
8_U87_MED1_5161_lociStitched	chr11	27864823	27914163
9_U87_MED1_2459_lociStitched	chr1	199680258	199726111
4_U87_MED1_7235_lociStitched	chr12	74697858	74717726
5_U87_MED1_4730_lociStitched	chr10	127900118	127932927
6_U87_MED1_22320_lociStitched	chr4	75178405	75219573
5_U87_MED1_7127_lociStitched	chr12	64537567	64575468
4_U87_MED1_17184_lociStitched	chr2	226993280	227021635
5_U87_MED1_16908_lociStitched	chr2	207958570	207998045
5_U87_MED1_15976_lociStitched	chr2	113096151	113124471
5_U87_MED1_19506_lociStitched	chr3	4727890	4764151
1_U87_MED1_204_lociStitched	chr1	11889850	11893062
3_U87_MED1_13789_lociStitched	chr19	1198622	1219360
5_U87_MED1_4735_lociStitched	chr10	128052098	128101696
5_U87_MED1_31406_lociStitched	chr9	122171758	122206963
5_U87_MED1_9188_lociStitched	chr14	76558717	76596203
2_U87_MED1_8973_lociStitched	chr14	64612804	64614687
11_U87_MED1_18186_lociStitched	chr20	49369237	49419385
2_U87_MED1_29678_lociStitched	chr8	99439088	99452526
4_U87_MED1_5551_lociStitched	chr11	61478002	61500583
10_U87_MED1_2485_lociStitched	chr1	199971787	200011527
6_U87_MED1_3459_lociStitched	chr10	29949767	29989251
7_U87_MED1_17938_lociStitched	chr20	36229309	36280924
6_U87_MED1_21893_lociStitched	chr4	13498370	13544429
5_U87_MED1_18845_lociStitched	chr21	46282572	46307112
5_U87_MED1_28712_lociStitched	chr8	11343022	11377910
5_U87_MED1_11837_lociStitched	chr17	13568637	13595972
5_U87_MED1_26443_lociStitched	chr6	112399077	112447095

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REGION_ID	CHROM	START	STOP
10_U87_MED1_27794_lociStitched	chr7	76875842	76933318
6_U87_MED1_3787_lociStitched	chr10	63974312	64024395
3_U87_MED1_32094_lociStitched	chrX	45249029	45269337
7_U87_MED1_2818_lociStitched	chr1	221956329	221986465
1_U87_MED1_7642_lociStitched	chr12	119212723	119216302
5_U87_MED1_15994_lociStitched	chr2	113341276	113362882
5_U87_MED1_20226_lociStitched	chr3	55462763	55499466
8_U87_MED1_25357_lociStitched	chr5	179683140	179713756
10_U87_MED1_21194_lociStitched	chr3	150772593	150825135
4_U87_MED1_19522_lociStitched	chr3	5033023	5054218
4_U87_MED1_15905_lociStitched	chr2	105378414	105401351
3_U87_MED1_17219_lociStitched	chr2	228388427	228408117
2_U87_MED1_13120_lociStitched	chr18	3436350	3456896
4_U87_MED1_3990_lociStitched	chr10	75315563	75338505
4_U87_MED1_11024_lociStitched	chr16	49738943	49767162
5_U87_MED1_770_lociStitched	chr1	36580667	36626685
4_U87_MED1_719_lociStitched	chr1	33647773	33674750
3_U87_MED1_24071_lociStitched	chr5	90698489	90717110
12_U87_MED1_5468_lociStitched	chr11	56798699	56850272
4_U87_MED1_15820_lociStitched	chr2	99841615	99866659
4_U87_MED1_12059_lociStitched	chr17	27024805	27048099
4_U87_MED1_19578_lociStitched	chr3	10205559	10223302
9_U87_MED1_31314_lociStitched	chr9	116906615	116969979
5_U87_MED1_17381_lociStitched	chr2	237695304	237731727
4_U87_MED1_15023_lociStitched	chr2	28463256	28486432
6_U87_MED1_16974_lociStitched	chr2	216253277	216287004
6_U87_MED1_16311_lociStitched	chr2	151031128	151061882
6_U87_MED1_3547_lociStitched	chr10	33659030	33711377
5_U87_MED1_11814_lociStitched	chr17	13181474	13210125
4_U87_MED1_28576_lociStitched	chr7	151008488	151029657
1_U87_MED1_13124_lociStitched	chr18	3611922	3616326
8_U87_MED1_3534_lociStitched	chr10	33444568	33494188
6_U87_MED1_1871_lociStitched	chr1	150209432	150241437
2_U87_MED1_18051_lociStitched	chr20	43832868	43845622
3_U87_MED1_10146_lociStitched	chr15	70301493	70317899
7_U87_MED1_2527_lociStitched	chr1	201747626	201796040
13_U87_MED1_24272_lociStitched	chr5	112383768	112458948
5_U87_MED1_26430_lociStitched	chr6	112137473	112179561
4_U87_MED1_13429_lociStitched	chr18	41626488	41662617
6_U87_MED1_8590_lociStitched	chr13	113882656	113916801
9_U87_MED1_9551_lociStitched	chr15	30738592	30802325
6_U87_MED1_17744_lociStitched	chr20	23071349	23090627
6_U87_MED1_10416_lociStitched	chr15	88161556	88193745
3_U87_MED1_5273_lociStitched	chr11	35007450	35019639
2_U87_MED1_1556_lociStitched	chr1	100859494	100870177
4_U87_MED1_29271_lociStitched	chr8	49481932	49508141
5_U87_MED1_19225_lociStitched	chr22	36029312	36057715
5_U87_MED1_29265_lociStitched	chr8	49377506	49400335
10_U87_MED1_9028_lociStitched	chr14	68199644	68255143
8_U87_MED1_12801_lociStitched	chr17	67895139	67931773
2_U87_MED1_4101_lociStitched	chr10	79683341	79694556
5_U87_MED1_16956_lociStitched	chr2	215974532	216011850
6_U87_MED1_32082_lociStitched	chrX	43746648	43786932
4_U87_MED1_18137_lociStitched	chr20	48353990	48372553
1_U87_MED1_5584_lociStitched	chr11	62363092	62367099
6_U87_MED1_15922_lociStitched	chr2	108226124	108262222
5_U87_MED1_16864_lociStitched	chr2	204370112	204385649
14_U87_MED1_16427_lociStitched	chr2	160916322	160997972
8_U87_MED1_23630_lociStitched	chr5	52329945	52369930
5_U87_MED1_17309_lociStitched	chr2	234814049	234832679
U87_MED1_12055	chr17	26929956	26934384
6_U87_MED1_23098_lociStitched	chr4	182794994	182847907
7_U87_MED1_2995_lociStitched	chr1	233157788	233200699
4_U87_MED1_4147_lociStitched	chr10	80745860	80764435
10_U87_MED1_25839_lociStitched	chr6	35221878	35273955
2_U87_MED1_3179_lociStitched	chr10	4794967	4808857
5_U87_MED1_12475_lociStitched	chr17	45628535	45655344
3_U87_MED1_28098_lociStitched	chr7	105697048	105714277
1_U87_MED1_23343_lociStitched	chr5	14157879	14165158
6_U87_MED1_20739_lociStitched	chr3	113836451	113858193
6_U87_MED1_2468_lociStitched	chr1	199766249	199799338
4_U87_MED1_4913_lociStitched	chr11	9730174	9767132
2_U87_MED1_20084_lociStitched	chr3	48567365	48579540
6_U87_MED1_28721_lociStitched	chr8	11390711	11411534
5_U87_MED1_7081_lociStitched	chr12	62839721	62868417
4_U87_MED1_23208_lociStitched	chr4	190929117	190951845

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REGION_ID	CHROM	START	STOP
8_U87_MED1_15178_lociStitched	chr2	37846146	37884311
5_U87_MED1_9939_lociStitched	chr15	60965417	60980962
9_U87_MED1_18605_lociStitched	chr21	35076849	35141236
7_U87_MED1_9763_lociStitched	chr15	46746824	46776787
3_U87_MED1_27564_lociStitched	chr7	45880224	45893741
5_U87_MED1_28912_lociStitched	chr8	23294269	23325787
2_U87_MED1_4059_lociStitched	chr10	78777531	78788869
5_U87_MED1_23069_lociStitched	chr4	178139337	178175485
3_U87_MED1_12646_lociStitched	chr17	56755482	56771755
3_U87_MED1_16239_lociStitched	chr2	143331502	143355637
3_U87_MED1_29002_lociStitched	chr8	26540715	26557275
6_U87_MED1_29815_lociStitched	chr8	116499299	116540088
6_U87_MED1_31373_lociStitched	chr9	118032147	118053805
4_U87_MED1_1780_lociStitched	chr1	144138664	144168151
5_U87_MED1_30166_lociStitched	chr8	132922317	132943207
9_U87_MED1_30246_lociStitched	chr8	134963771	135009147
3_U87_MED1_23445_lociStitched	chr5	33334716	33357539
6_U87_MED1_17250_lociStitched	chr2	230173938	230207111
5_U87_MED1_18788_lociStitched	chr21	43737139	43761842
6_U87_MED1_26457_lociStitched	chr6	112629871	112666312
6_U87_MED1_12208_lociStitched	chr17	35930846	35971407
9_U87_MED1_28935_lociStitched	chr8	23632043	23677190
6_U87_MED1_15610_lociStitched	chr2	72004300	72031901
4_U87_MED1_28289_lociStitched	chr7	128254122	128269877
1_U87_MED1_5182_lociStitched	chr11	28810629	28817709
2_U87_MED1_18609_lociStitched	chr21	35174645	35187060
5_U87_MED1_28350_lociStitched	chr7	130960595	130990571
4_U87_MED1_22927_lociStitched	chr4	158071384	158094259
5_U87_MED1_4012_lociStitched	chr10	76826281	76861798
7_U87_MED1_379_lociStitched	chr1	19621887	19652224
6_U87_MED1_18585_lociStitched	chr21	34818327	34848489
5_U87_MED1_904_lociStitched	chr1	43160882	43182066
2_U87_MED1_15586_lociStitched	chr2	70676208	70689547
2_U87_MED1_31394_lociStitched	chr9	118343216	118354200
1_U87_MED1_23352_lociStitched	chr5	14316952	14324472
10_U87_MED1_19673_lociStitched	chr3	14426598	14490180
6_U87_MED1_4067_lociStitched	chr10	78929913	78962884
4_U87_MED1_29950_lociStitched	chr8	123509564	123529619
4_U87_MED1_31528_lociStitched	chr9	129297192	129326262
4_U87_MED1_20561_lociStitched	chr3	100091920	100125071
5_U87_MED1_28581_lociStitched	chr7	151055255	151084698
5_U87_MED1_26426_lociStitched	chr6	111980027	112035051
2_U87_MED1_18956_lociStitched	chr22	23149440	23163217
2_U87_MED1_1656_lociStitched	chr1	112077002	112088768
4_U87_MED1_15603_lociStitched	chr2	71956835	71971756
3_U87_MED1_30321_lociStitched	chr8	145079732	145099991
3_U87_MED1_233_lociStitched	chr1	12575481	12603692
6_U87_MED1_28749_lociStitched	chr8	13254372	13279984
7_U87_MED1_1977_lociStitched	chr1	154332675	154367183
2_U87_MED1_18293_lociStitched	chr20	56022630	56028783
9_U87_MED1_886_lociStitched	chr1	41966619	42023301
6_U87_MED1_16981_lociStitched	chr2	216300355	216347664
6_U87_MED1_28927_lociStitched	chr8	23451758	23481764
1_U87_MED1_30073_lociStitched	chr8	128932139	128937025
5_U87_MED1_19816_lociStitched	chr3	27537533	27571776
7_U87_MED1_7805_lociStitched	chr12	126597650	126639572
5_U87_MED1_25946_lociStitched	chr6	43985976	44003858
3_U87_MED1_28109_lociStitched	chr7	105844701	105854365
2_U87_MED1_9252_lociStitched	chr14	89810151	89818908
4_U87_MED1_27267_lociStitched	chr7	22723409	22739542
13_U87_MED1_28793_lociStitched	chr8	19068482	19131291
6_U87_MED1_5481_lociStitched	chr11	56930199	56959561
2_U87_MED1_27568_lociStitched	chr7	45915902	45931369
6_U87_MED1_4303_lociStitched	chr10	95208065	95226275
7_U87_MED1_7132_lociStitched	chr12	64596525	64639788
6_U87_MED1_16065_lociStitched	chr2	121175738	121225198
U87_MED1_14366	chr19	47304243	47311641
7_U87_MED1_18808_lociStitched	chr21	43994975	44024520
2_U87_MED1_5008_lociStitched	chr11	12259582	12267357
4_U87_MED1_26112_lociStitched	chr6	56306428	56344388
2_U87_MED1_3174_lociStitched	chr10	4694138	4705791
7_U87_MED1_17815_lociStitched	chr20	29747030	29779683
4_U87_MED1_8309_lociStitched	chr13	79502139	79529052
8_U87_MED1_15724_lociStitched	chr2	84968849	85007114
5_U87_MED1_24412_lociStitched	chr5	131448786	131468778
6_U87_MED1_3854_lociStitched	chr10	69512331	69537255

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REGION_ID	CHROM	START	STOP
4_U87_MED1_14415_lociStitched	chr19	49931469	49950265
5_U87_MED1_14037_lociStitched	chr19	13121190	13144815
4_U87_MED1_7978_lociStitched	chr13	32722777	32758954
6_U87_MED1_8934_lociStitched	chr14	60998858	61027173
1_U87_MED1_31355_lociStitched	chr9	117490731	117497452
4_U87_MED1_16010_lociStitched	chr2	113713570	113730597
8_U87_MED1_29905_lociStitched	chr8	120625584	120684952
1_U87_MED1_12621_lociStitched	chr17	55214356	55220009
4_U87_MED1_18033_lociStitched	chr20	43105683	43130852
1_U87_MED1_14566_lociStitched	chr19	56760348	56770942
3_U87_MED1_6635_lociStitched	chr12	26157496	26179828
10_U87_MED1_26800_lociStitched	chr6	148859778	148930005
11_U87_MED1_3404_lociStitched	chr10	24761351	24796199
U87_MED1_6149	chr11	121571509	121574883
4_U87_MED1_30210_lociStitched	chr8	134368437	134385618
6_U87_MED1_1544_lociStitched	chr1	99882905	99924721
5_U87_MED1_12392_lociStitched	chr17	42688819	42727303
8_U87_MED1_20455_lociStitched	chr3	72114549	72164267
9_U87_MED1_28371_lociStitched	chr7	133767195	133816793
4_U87_MED1_1833_lociStitched	chr1	148842282	148859888
1_U87_MED1_16194_lociStitched	chr2	134260935	134266764
5_U87_MED1_3298_lociStitched	chr10	14467377	14497715
5_U87_MED1_19494_lociStitched	chr3	4417975	4444229
5_U87_MED1_23525_lociStitched	chr5	37806374	37829663
7_U87_MED1_20638_lociStitched	chr3	103127775	103167026
5_U87_MED1_15026_lociStitched	chr2	28518271	28546447
5_U87_MED1_24346_lociStitched	chr5	121505170	121548141
1_U87_MED1_72_lociStitched	chr1	7279930	7284880
2_U87_MED1_22344_lociStitched	chr4	75583830	75590802
2_U87_MED1_19612_lociStitched	chr3	11295015	11308874
5_U87_MED1_6644_lociStitched	chr12	26315522	26344028
4_U87_MED1_18578_lociStitched	chr21	34262111	34276116
3_U87_MED1_16960_lociStitched	chr2	216100277	216111305
3_U87_MED1_11901_lociStitched	chr17	16984314	17001391
3_U87_MED1_5664_lociStitched	chr11	65079515	65090536
4_U87_MED1_14346_lociStitched	chr19	46416158	46427894
2_U87_MED1_24022_lociStitched	chr5	86448382	86461105
3_U87_MED1_12721_lociStitched	chr17	61694793	61709067
6_U87_MED1_24200_lociStitched	chr5	106725129	106753981
11_U87_MED1_25306_lociStitched	chr5	177709555	177748817
7_U87_MED1_13705_lociStitched	chr18	66175119	66216988
5_U87_MED1_14892_lociStitched	chr2	20229043	20250537
5_U87_MED1_358_lociStitched	chr1	18060509	18078661
8_U87_MED1_29868_lociStitched	chr8	119059307	119101868
2_U87_MED1_31353_lociStitched	chr9	117468982	117476962
4_U87_MED1_26509_lociStitched	chr6	117867944	117880133
4_U87_MED1_6791_lociStitched	chr12	45948501	45963507
1_U87_MED1_7316_lociStitched	chr12	88263372	88272888
1_U87_MED1_28454_lociStitched	chr7	139014545	139019742
3_U87_MED1_29676_lociStitched	chr8	99413377	99423302
6_U87_MED1_23651_lociStitched	chr5	52728355	52762900
4_U87_MED1_29126_lociStitched	chr8	32294513	32321547
1_U87_MED1_16937_lociStitched	chr2	213414737	213420189
5_U87_MED1_9017_lociStitched	chr14	68071320	68092309
2_U87_MED1_7050_lociStitched	chr12	61281616	61290216
2_U87_MED1_8479_lociStitched	chr13	105600869	105608876
3_U87_MED1_28991_lociStitched	chr8	26361671	26378373
9_U87_MED1_3200_lociStitched	chr10	5566940	5627226
3_U87_MED1_30976_lociStitched	chr9	96662387	96672240
4_U87_MED1_29291_lociStitched	chr8	49966143	50006378
6_U87_MED1_9106_lociStitched	chr14	72173702	72203999
8_U87_MED1_16377_lociStitched	chr2	158024751	158054345
5_U87_MED1_21864_lociStitched	chr4	9775313	9798168
4_U87_MED1_11370_lociStitched	chr16	75998735	76031950
7_U87_MED1_15218_lociStitched	chr2	39560078	39595092
8_U87_MED1_12696_lociStitched	chr17	59756987	59812906
8_U87_MED1_25005_lociStitched	chr5	159233084	159273365
2_U87_MED1_14996_lociStitched	chr2	27872924	27887411
1_U87_MED1_9389_lociStitched	chr14	96712814	96717531
5_U87_MED1_27903_lociStitched	chr7	92088104	92112431
7_U87_MED1_4679_lociStitched	chr10	124239479	124271216
6_U87_MED1_30005_lociStitched	chr8	125807278	125854903
6_U87_MED1_28456_lociStitched	chr7	139069397	139084495
2_U87_MED1_24155_lociStitched	chr5	97670802	97679322
8_U87_MED1_21597_lociStitched	chr3	189462566	189498548
6_U87_MED1_11827_lociStitched	chr17	13364635	13408579

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REGION_ID	CHROM	START	STOP
7_U87_MED1_3680_lociStitched	chr10	50039199	50063569
2_U87_MED1_19061_lociStitched	chr22	29134450	29153278
3_U87_MED1_23518_lociStitched	chr5	37750719	37760798
3_U87_MED1_7310_lociStitched	chr12	88073577	88084969
1_U87_MED1_13590_lociStitched	chr18	54176974	54185409
8_U87_MED1_8053_lociStitched	chr13	42279138	42321467
4_U87_MED1_12184_lociStitched	chr17	35505423	35524108
4_U87_MED1_13264_lociStitched	chr18	18384075	18398874
2_U87_MED1_9121_lociStitched	chr14	72995992	73005780
1_U87_MED1_318_lociStitched	chr1	16712459	16713704
4_U87_MED1_28428_lociStitched	chr7	136991184	137032443
1_U87_MED1_21899_lociStitched	chr4	13595377	13602846
6_U87_MED1_1236_lociStitched	chr1	66500300	66532014
2_U87_MED1_23649_lociStitched	chr5	52691426	52701566
2_U87_MED1_1146_lociStitched	chr1	59278815	59285007
3_U87_MED1_4738_lociStitched	chr10	128136077	128148428
1_U87_MED1_16895_lociStitched	chr2	207733794	207741019
U87_MED1_6147	chr11	121541171	121547835
8_U87_MED1_18611_lociStitched	chr21	35259187	35300591
5_U87_MED1_21690_lociStitched	chr3	195673864	195696713
6_U87_MED1_24681_lociStitched	chr5	142535854	142577824
4_U87_MED1_25215_lociStitched	chr5	172809327	172835222
4_U87_MED1_14706_lociStitched	chr2	9220694	9250055
5_U87_MED1_17204_lociStitched	chr2	227939599	227962404
8_U87_MED1_28194_lociStitched	chr7	115849254	115893839
5_U87_MED1_19772_lociStitched	chr3	23665020	23703368
3_U87_MED1_15004_lociStitched	chr2	28029902	28039153
4_U87_MED1_106_lociStitched	chr1	8103590	8124963
5_U87_MED1_817_lociStitched	chr1	39625358	39648692
6_U87_MED1_28612_lociStitched	chr7	154678056	154722945
7_U87_MED1_432_lociStitched	chr1	21495634	21538118
2_U87_MED1_31267_lociStitched	chr9	115421719	115435645
7_U87_MED1_12576_lociStitched	chr17	53290132	53326319
6_U87_MED1_19801_lociStitched	chr3	25588763	25621509
3_U87_MED1_27050_lociStitched	chr7	183514	197682
9_U87_MED1_14480_lociStitched	chr19	52127006	52186848
6_U87_MED1_19418_lociStitched	chr22	44358335	44388085
2_U87_MED1_30163_lociStitched	chr8	132894337	132909622
U87_MED1_14367	chr19	47316482	47321267
4_U87_MED1_12273_lociStitched	chr17	37922830	37933478
5_U87_MED1_24689_lociStitched	chr5	142592522	142623091
4_U87_MED1_20269_lociStitched	chr3	58004870	58021097
3_U87_MED1_27523_lociStitched	chr7	43645607	43666145
1_U87_MED1_17177_lociStitched	chr2	226687043	226693005
2_U87_MED1_6543_lociStitched	chr12	13239108	13252365
5_U87_MED1_17087_lociStitched	chr2	220013772	220043266
1_U87_MED1_30069_lociStitched	chr8	128815091	128825309
4_U87_MED1_29371_lociStitched	chr8	59816874	59844700
1_U87_MED1_6537_lociStitched	chr12	13141485	13148287
7_U87_MED1_5290_lociStitched	chr11	35188819	35225440
2_U87_MED1_27570_lociStitched	chr7	45982964	45992590
2_U87_MED1_14283_lociStitched	chr19	43180211	43188202
4_U87_MED1_15878_lociStitched	chr2	101801067	101827913
7_U87_MED1_3818_lociStitched	chr10	64389081	64435800
1_U87_MED1_1809_lociStitched	chr1	148122343	148127505
7_U87_MED1_4527_lociStitched	chr10	112142380	112177089
3_U87_MED1_779_lociStitched	chr1	37709463	37726142
3_U87_MED1_28080_lociStitched	chr7	104399029	104413643
8_U87_MED1_18902_lociStitched	chr22	19188215	19237227
2_U87_MED1_16544_lociStitched	chr2	173720790	173735078
3_U87_MED1_14022_lociStitched	chr19	12749117	12766578
4_U87_MED1_1829_lociStitched	chr1	148799756	148819583
3_U87_MED1_1539_lociStitched	chr1	99827254	99845083
3_U87_MED1_4484_lociStitched	chr10	106077029	106101948
5_U87_MED1_30974_lociStitched	chr9	96582128	96608518
5_U87_MED1_8539_lociStitched	chr13	109840351	109863235
2_U87_MED1_21146_lociStitched	chr3	147358174	147368300
3_U87_MED1_13125_lociStitched	chr18	3638374	3656877
6_U87_MED1_24416_lociStitched	chr5	131578682	131630139
6_U87_MED1_27818_1ociStitched	chr7	80166238	80194389
4_U87_MED1_31023_lociStitched	chr9	100662763	100685315
2_U87_MED1_15229_lociStitched	chr2	40176624	40187049

TABLE 4

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REGION_ID	CHROM	START	STOP
1_H2171_MED1_1_1640_lociStitched	chr12	6920935	6927602
3_H2171_MED1_1_4743_lociStitched	chr20	20467079	20497912
7_H2171_MED1_1_1324_lociStitched	chr11	44999379	45032693
7_H2171_MED1_1_4739_lociStitched	chr20	20368291	20422337
3_H2171_MED1_1_4728_lociStitched	chr20	20127551	20146821
5_H2171_MED1_1_2525_lociStitched	chr14	100006544	100041089
10_H2171_MED1_1_1318_lociStitched	chr11	44914282	44976798
7_H2171_MED1_1_3367_lociStitched	chr17	52974161	53020737
5_H2171_MED1_1_2568_lociStitched	chr14	105386944	105407220
4_H2171_MED1_1_2193_lociStitched	chr13	70984696	70997790
4_H2171_MED1_1_1411_lociStitched	chr11	65001189	65034088
5_H2171_MED1_1_2727_lociStitched	chr15	67058222	67081109
4_H2171_MED1_1_4448_lociStitched	chr2	182187487	182216832
2_H2171_MED1_1_3306_lociStitched	chr17	38792864	38802484
7_H2171_MED1_1_3117_lociStitched	chr16	84027236	84077758
4_H2171_MED1_1_2523_lociStitched	chr14	99952877	99984071
4_H2171_MED1_1_6398_lociStitched	chr6	20798985	20817496
1_H2171_MED1_1_5368_lociStitched	chr3	73242222	73243091
2_H2171_MED1_1_1409_lociStitched	chr11	64938799	64950566
10_H2171_MED1_1_5063_lociStitched	chr22	28420926	28471660
3_H2171_MED1_1_1518_lociStitched	chr11	110675092	110687227
2_H2171_MED1_1_106_lociStitched	chr1	17094879	17105111
7_H2171_MED1_1_370_lociStitched	chr1	61124688	61164318
3_H2171_MED1_1_4670_lociStitched	chr20	5763423	5778470
3_H2171_MED1_1_2458_lociStitched	chr14	80493803	80524114
2_H2171_MED1_1_2703_lociStitched	chr15	63374895	63384854
2_H2171_MED1_1_196_lociStitched	chr1	27718317	27729348
1_H2171_MED1_1_1626_lociStitched	chr12	1909405	1917933
1_H2171_MED1_1_2022_lociStitched	chr12	119212791	119216166
7_H2171_MED1_1_2994_lociStitched	chr16	48115499	48154218
1_H2171_MED1_1_1385_lociStitched	chr11	62364199	62367040
3_H2171_MED1_1_355_lociStitched	chr1	60460911	60473852
8_H2171_MED1_1_4077_lociStitched	chr2	50900527	50957040
3_H2171_MED1_1_4992_lociStitched	chr21	45354314	45373451
3_H2171_MED1_1_4776_lociStitched	chr20	29744744	29765111
1_H2171_MED1_1_86_lociStitched	chr1	11890040	11892976
1_H2171_MED1_1_4772_lociStitched	chr20	29655198	29660784
1_H2171_MED1_1_1806_lociStitched	chr12	55914077	55924333
6_H2171_MED1_1_4832_lociStitched	chr20	44860383	44878078
7_H2171_MED1_1_2352_lociStitched	chr14	54625929	54653893
4_H2171_MED1_1_2589_lociStitched	chr15	29345063	29360788
9_H2171_MED1_1_1076_lociStitched	chr10	80658480	80712619
2_H2171_MED1_1_6438_lociStitched	chr6	26263284	26281349
5_H2171_MED1_1_4748_lociStitched	chr20	20518980	20554248
3_H2171_MED1_1_1797_lociStitched	chr12	53731066	53749016
5_H2171_MED1_1_259_lociStitched	chr1	41603873	41629260
2_H2171_MED1_1_4451_lociStitched	chr2	182245805	182255349
7_H2171_MED1_1_4066_lociStitched	chr2	50831888	50874042
3_H2171_MED1_1_1331_lociStitched	chr11	45063502	45081811
3_H2171_MED1_1_7960_lociStitched	chr9	131283833	131300537
1_H2171_MED1_1_3376_lociStitched	chr17	54062985	54065019
2_H2171_MED1_1_3964_lociStitched	chr2	8734984	8744081
1_H2171_MED1_1_844_lociStitched	chr1	232925154	232930496
2_H2171_MED1_1_3925_lociStitched	chr2	2305821	2317044
1_H2171_MED1_1_7716_lociStitched	chr9	72222711	72226329
1_H2171_MED1_1_3377_lociStitched	chr17	54090881	54092427
1_H2171_MED1_1_2879_lociStitched	chr16	2456826	2462820
3_H2171_MED1_1_2486_lociStitched	chr14	90046046	90059450
3_H2171_MED1_1_6363_lociStitched	chr6	17580996	17600893
4_H2171_MED1_1_2646_lociStitched	chr15	44378691	44396308
3_H2171_MED1_1_7981_lociStitched	chr9	133669354	133683692
5_H2171_MED1_1_7401_lociStitched	chr8	63107445	63135528
2_H2171_MED1_1_873_lociStitched	chr1	241942356	241953358
1_H2171_MED1_1_1285_lociStitched	chr11	31851717	31855125
4_H2171_MED1_1_7215_lociStitched	chr7	127256053	127272711
4_H2171_MED1_1_2597_lociStitched	chr15	29404315	29442687
2_H2171_MED1_1_4761_lociStitched	chr20	24646465	24652204
3_H2171_MED1_1_4781_lociStitched	chr20	30575765	30589140
2_H2171_MED1_1_3111_lociStitched	chr16	83975326	83989531
4_H2171_MED1_1_488_lociStitched	chr1	116596631	116613122
3_H2171_MED1_1_7399_lociStitched	chr8	62993333	63023912
4_H2171_MED1_1_4597_lociStitched	chr2	232245138	232257967
5_H2171_MED1_1_1703_lociStitched	chr12	28479296	28497904
4_H2171_MED1_1_8014_lociStitched	chr9	136814438	136833329
2_H2171_MED1_1_2533_lociStitched	chr14	100108519	100126673
5_H2171_MED1_1_6671_lociStitched	chr6	112078789	112091156

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REGION_ID	CHROM	START	STOP
3_H2171_MED1_1_6930_lociStitched	chr7	31684707	31699272
2_H2171_MED1_1_277_lociStitched	chr1	44959548	44969924
1_H2171_MED1_1_4770_lociStitched	chr20	29623515	29626066
3_H2171_MED1_1_3229_lociStitched	chr17	18824205	18838508
5_H2171_MED1_1_7373_lociStitched	chr8	53305190	53330760
2_H2171_MED1_1_4445_lociStitched	chr2	182146929	182160614
5_H2171_MED1_1_6182_lociStitched	chr5	142369672	142397549
3_H2171_MED1_1_3109_lociStitched	chr16	83939477	83957133
1_H2171_MED1_1_6436_lociStitched	chr6	26230266	26234969
2_H2171_MED1_1_5573_lociStitched	chr3	171666644	171672601
4_H2171_MED1_1_7990_lociStitched	chr9	133870805	133889409
7_H2171_MED1_1_324_lociStitched	chr1	54535842	54595884
3_H2171_MED1_1_4733_lociStitched	chr20	20330857	20340022
1_H2171_MED1_1_1286_lociStitched	chr11	31970692	31975143
3_H2171_MED1_1_6477_lociStitched	chr6	33819339	33828849
4_H2171_MED1_1_5144_lociStitched	chr22	41520431	41540832
2_H2171_MED1_1_5576_lociStitched	chr3	171727766	171734092
3_H2171_MED1_1_7552_lociStitched	chr8	125856085	125872149
3_H2171_MED1_1_7535_lociStitched	chr8	123754555	123765925
3_H2171_MED1_1_5948_lociStitched	chr5	14793111	14810119
2_H2171_MED1_1_5868_lociStitched	chr4	141377946	141394403
2_H2171_MED1_1_1526_lociStitched	chr11	110802193	110813715
1_H2171_MED1_1_3506_lociStitched	chr17	75396929	75402414
4_H2171_MED1_1_4283_lociStitched	chr2	134996095	135011003
3_H2171_MED1_1_6663_lociStitched	chr6	111978600	111995752
3_H2171_MED1_1_858_lociStitched	chr1	235546011	235556631
1_H2171_MED1_1_3207_lociStitched	chr17	8016708	8018589
4_H2171_MED1_1_303_lociStitched	chr1	53346865	53379175
3_H2171_MED1_1_6854_lociStitched	chr7	3273583	3282459
4_H2171_MED1_1_2201_lociStitched	chr13	71269244	71287635
2_H2171_MED1_1_5514_lociStitched	chr3	141542495	141547705
1_H2171_MED1_1_102_lociStitched	chr1	16712502	16713836
1_H2171_MED1_1_3304_lociStitched	chr17	38747760	38749588
4_H2171_MED1_1_3851_lociStitched	chr19	43240289	43257408
3_H2171_MED1_1_7984_lociStitched	chr9	133750060	133767255
2_H2171_MED1_1_2593_lociStitched	chr15	29374833	29382092
4_H2171_MED1_1_1632_lociStitched	chr12	3191844	3208689
3_H2171_MED1_1_613_lociStitched	chr1	181446125	181455812
1_H2171_MED1_1_515_lociStitched	chr1	147489769	147491715
1_H2171_MED1_1_7564_lociStitched	chr8	127859208	127871721
2_H2171_MED1_1_4141_lociStitched	chr2	70212694	70224525
1_H2171_MED1_1_3928_lociStitched	chr2	2827367	2830692
3_H2171_MED1_1_3104_lociStitched	chr16	83865721	83879079
3_H2171_MED1_1_7998_lociStitched	chr9	134078841	134097047
3_H2171_MED1_1_7465_lociStitched	chr8	93687205	93693913
3_H2171_MED1_1_2733_lociStitched	chr15	67212499	67237556
4_H2171_MED1_1_2715_lociStitched	chr15	64230622	64250211
2_H2171_MED1_1_1770_lociStitched	chr12	48729670	48733984
5_H2171_MED1_1_5251_lociStitched	chr3	16817691	16841285
2_H2171_MED1_1_8026_lociStitched	chr9	137161098	137170211
4_H2171_MED1_1_5775_lociStitched	chr4	80519421	80536721
3_H2171_MED1_1_4835_lociStitched	chr20	45030700	45042538
2_H2171_MED1_1_5461_lociStitched	chr3	127738334	127747382
1_H2171_MED1_1_3360_lociStitched	chr17	52949374	52952330
3_H2171_MED1_1_4792_lociStitched	chr20	31606072	31629076
3_H2171_MED1_1_5099_lociStitched	chr22	36154719	36175974
3_H2171_MED1_1_3453_lociStitched	chr17	69839454	69850658
2_H2171_MED1_1_606_lociStitched	chr1	180846204	180855565
3_H2171_MED1_1_2977_lociStitched	chr16	47549993	47564543
5_H2171_MED1_1_5509_lociStitched	chr3	141387464	141408827
4_H2171_MED1_1_6832_lociStitched	chr7	1281279	1305738
3_H2171_MED1_1_7419_1ociStitched	chr8	64128807	64152294
3_H2171_MED1_1_392_lociStitched	chr1	67883445	67891831
3_H2171_MED1_1_7851_lociStitched	chr9	111073312	111081518
1_H2171_MED1_1_3482_lociStitched	chr17	73307672	73311962
1_H2171_MED1_1_677_lociStitched	chr1	200341028	200344953
4_H2171_MED1_1_2770_lociStitched	chr15	72303789	72325673
1_H2171_MED1_1_1390_lociStitched	chr11	63440196	63445356
5_H2171_MED1_1_5682_lociStitched	chr4	8071726	8098132
2_H2171_MED1_1_358_lociStitched	chr1	60514461	60520511
2_H2171_MED1_1_8028_lociStitched	chr9	137386784	137396443
4_H2171_MED1_1_4955_lociStitched	chr21	38139981	38165165
6_H2171_MED1_1_1142_lociStitched	chr10	112592513	112615109
4_H2171_MED1_1_672_lociStitched	chr1	200253434	200274407
2_H2171_MED1_1_1016_lociStitched	chr10	73690165	73706428
3_H2171_MED1_1_5762_lociStitched	chr4	80338728	80352494

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REGION_ID	CHROM	START	STOP
2_H2171_MED1_1_6852_lociStitched	chr7	3187356	3195840
2_H2171_MED1_1_4985_lociStitched	chr21	44518734	44524811
3_H2171_MED1_1_4943_lociStitched	chr21	33430682	33447308
1_H2171_MED1_1_3368_lociStitched	chr17	53035354	53040644
4_H2171_MED1_1_2427_lociStitched	chr14	76442631	76461700
4_H2171_MED1_1_4558_lociStitched	chr2	217169924	217196187
3_H2171_MED1_1_7744_lociStitched	chr9	85109090	85122979
5_H2171_MED1_1_1691_lociStitched	chr12	28264210	28281514
3_H2171_MED1_1_2892_lociStitched	chr16	11047692	11059849
2_H2171_MED1_1_4899_lociStitched	chr20	60880400	60885360
1_H2171_MED1_1_7947_lociStitched	chr9	129461873	129464518
2_H2171_MED1_1_674_lociStitched	chr1	200292057	200306846
2_H2171_MED1_1_2203_lociStitched	chr13	71325559	71338429
2_H2171_MED1_1_3186_lociStitched	chr17	3729010	3741942
2_H2171_MED1_1_2909_lociStitched	chr16	11781980	11794751
2_H2171_MED1_1_4766_lociStitched	chr20	25613739	25620530
3_H2171_MED1_1_2897_lociStitched	chr16	11144367	11154452
1_H2171_MED1_1_5910_lociStitched	chr5	451422	454266
4_H2171_MED1_1_7415_lociStitched	chr8	63776687	63806155
1_H2171_MED1_1_1453_lociStitched	chr11	78328712	78331262
3_H2171_MED1_1_6678_lociStitched	chr6	112348676	112356273
2_H2171_MED1_1_1765_lociStitched	chr12	48546951	48555151
1_H2171_MED1_1_2433_lociStitched	chr14	76568042	76570777
1_H2171_MED1_1_7993_lociStitched	chr9	133904790	133908837
2_H2171_MED1_1_3164_lociStitched	chr17	1642357	1647888
4_H2171_MED1_1_1653_lociStitched	chr12	8501768	8523291
2_H2171_MED1_1_8030_lociStitched	chr9	138135585	138142078
4_H2171_MED1_1_6697_lociStitched	chr6	114858043	114874406
2_H2171_MED1_1_2731_lociStitched	chr15	67146253	67153108
1_H2171_MED1_1_676_lociStitched	chr1	200320223	200323463
3_H2171_MED1_1_7106_lociStitched	chr7	90891248	90904646
3_H2171_MED1_1_1282_lociStitched	chr11	31605924	31622763
1_H2171_MED1_1_6439_lociStitched	chr6	26304930	26308840
3_H2171_MED1_1_6542_lociStitched	chr6	43874271	43891627
2_H2171_MED1_1_362_lociStitched	chr1	61095855	61105484
3_H2171_MED1_1_4288_lociStitched	chr2	135067807	135078675
1_H2171_MED1_1_2649_lociStitched	chr15	44422399	44425293
4_H2171_MED1_1_1859_lociStitched	chr12	74259275	74282803
2_H2171_MED1_1_6347_lociStitched	chr6	15255876	15261598
3_H2171_MED1_1_3953_lociStitched	chr2	7321393	7328314
1_H2171_MED1_1_3540_lociStitched	chr17	77837805	77840024
2_H2171_MED1_1_6860_lociStitched	chr7	5429395	5446166
2_H2171_MED1_1_3999_lociStitched	chr2	23567475	23572888
1_H2171_MED1_1_5475_lociStitched	chr3	130776820	130781469
1_H2171_MED1_1_4528_lociStitched	chr2	207882381	207885158
2_H2171_MED1_1_842_lociStitched	chr1	232900985	232903673
2_H2171_MED1_1_3986_lociStitched	chr2	20412482	20420065
2_H2171_MED1_1_3326_lociStitched	chr17	43434796	43447830
3_H2171_MED1_1_5929_lociStitched	chr5	8720521	8737555
2_H2171_MED1_1_4569_lociStitched	chr2	218968606	218980938
3_H2171_MED1_1_890_lociStitched	chr10	1486532	1493897
1_H2171_MED1_1_7959_lociStitched	chr9	131260227	131263591
3_H2171_MED1_1_2166_lociStitched	chr13	58914764	58925684
1_H2171_MED1_1_8018_lociStitched	chr9	136926589	136930226
4_H2171_MED1_1_3837_lociStitched	chr19	40215562	40239946
2_H2171_MED1_1_6546_lociStitched	chr6	43906068	43912657
1_H2171_MED1_1_331_lociStitched	chr1	54796212	54799014
2_H2171_MED1_1_1637_lociStitched	chr12	3677173	3687680
2_H2171_MED1_1_4907_lociStitched	chr20	61600274	61610725
1_H2171_MED1_1_4771_lociStitched	chr20	29638614	29640239
1_H2171_MED1_1_6475_lociStitched	chr6	33043033	33048720
4_H2171_MED1_1_6405_lociStitched	chr6	21296380	21310734
2_H2171_MED1_1_5758_lociStitched	chr4	80183595	80191861
1_H2171_MED1_1_7556_lociStitched	chr8	126466494	126468843
2_H2171_MED1_1_4002_lociStitched	chr2	23606108	23613290
2_H2171_MED1_1_2058_lociStitched	chr12	123805217	123810213
2_H2171_MED1_1_849_lociStitched	chr1	233312827	233321459
3_H2171_MED1_1_3899_lociStitched	chr19	53527025	53546826
2_H2171_MED1_1_395_lociStitched	chr1	67912539	67916665
4_H2171_MED1_1_6138_lociStitched	chr5	134851883	134865995
2_H2171_MED1_1_4060_lociStitched	chr2	50612904	50617830
2_H2171_MED1_1_4365_lociStitched	chr2	155710278	155720040
2_H2171_MED1_1_7889_lociStitched	chr9	119201834	119207212
1_H2171_MED1_1_7587_lociStitched	chr8	134455564	134458775
3_H2171_MED1_1_2601_lociStitched	chr15	29458295	29478793
3_H2171_MED1_1_1260_lociStitched	chr11	22313265	22322600

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REGION_ID	CHROM	START	STOP
3_H2171_MED1_1_5218_lociStitched	chr3	10469904	10487109
3_H2171_MED1_1_6827_lociStitched	chr7	1204071	1217969
5_H2171_MED1_1_2046_lociStitched	chr12	123556670	123583312
2_H2171_MED1_1_193_lociStitched	chr1	27053645	27065430
2_H2171_MED1_1_4672_lociStitched	chr20	5798497	5808334
4_H2171_MED1_1_1335_lociStitched	chr11	45327480	45350583
2_H2171_MED1_1_3922_lociStitched	chr2	1989631	2000522
3_H2171_MED1_1_1783_lociStitched	chr12	51552711	51560557
1_H2171_MED1_1_4572_lociStitched	chr2	219568763	219574266
1_H2171_MED1_1_8038_lociStitched	chr9	138634973	138640138
2_H2171_MED1_1_450_lociStitched	chr1	107836814	107840728
1_H2171_MED1_1_6340_lociStitched	chr6	15092300	15095311
1_H2171_MED1_1_3904_lociStitched	chr19	53829063	53833837
5_H2171_MED1_1_5224_lociStitched	chr3	11299162	11324095
2_H2171_MED1_1_5986_lociStitched	chr5	35395346	35406891
3_H2171_MED1_1_1693_lociStitched	chr12	28296478	28309575
1_H2171_MED1_1_5922_lociStitched	chr5	3542098	3546382
3_H2171_MED1_1_2604_lociStitched	chr15	29544679	29560827
2_H2171_MED1_1_2254_lociStitched	chr13	99297247	99306503
4_H2171_MED1_1_2041_lociStitched	chr12	123445277	123474714
2_H2171_MED1_1_2141_lociStitched	chr13	52466506	52478601
6_H2171_MED1_1_817_lociStitched	chr1	230727775	230758255
3_H2171_MED1_1_2411_lociStitched	chr14	73925775	73939983
1_H2171_MED1_1_6346_lociStitched	chr6	15239481	15242062
2_H2171_MED1_1_31_lociStitched	chr1	6252175	6261523
2_H2171_MED1_1_6334_lociStitched	chr6	14651962	14658490
1_H2171_MED1_1_10_lociStitched	chr1	1355263	1360155
1_H2171_MED1_1_534_lociStitched	chr1	153237908	153244271
1_H2171_MED1_1_3988_lociStitched	chr2	20600392	20605469
1_H2171_MED1_1_5909_lociStitched	chr5	423764	428358
1_H2171_MED1_1_4170_lociStitched	chr2	86115761	86118411
1_H2171_MED1_1_7411_lociStitched	chr8	63579148	63586116
3_H2171_MED1_1_2624_lociStitched	chr15	37633152	37638629
1_H2171_MED1_1_5911_lociStitched	chr5	695232	697860
3_H2171_MED1_1_1253_lociStitched	chr11	19713467	19724753
3_H2171_MED1_1_2541_lociStitched	chr14	100247113	100254243
2_H2171_MED1_1_7301_lociStitched	chr7	157275487	157282112
1_H2171_MED1_1_5103_lociStitched	chr22	36214156	36218182
3_H2171_MED1_1_3372_lociStitched	chr17	53332524	53350328
2_H2171_MED1_1_2657_lociStitched	chr15	45586454	45593969
2_H2171_MED1_1_4694_lociStitched	chr20	12456692	12465019
2_H2171_MED1_1_6684_lociStitched	chr6	112571616	112582817
1_H2171_MED1_1_3996_lociStitched	chr2	22829298	22831811
2_H2171_MED1_1_8040_lociStitched	chr9	138704382	138711169
1_H2171_MED1_1_5920_lociStitched	chr5	3445869	3449196
1_H2171_MED1_1_5760_lociStitched	chr4	80303607	80306058

What is claimed is:

1. A method of identifying a super-enhancer in a sample comprising chromatin, comprising: obtaining chromatin from a cell wherein said chromatin has been cross-linked such that chromosomal nucleic acid in the chromatin is cross-linked to a component selected from the group consisting of BRD4, a Mediator component and H3K27Ac with which the chromosomal nucleic acid is associated to form a cross-linked complex; contacting said cross-linked complex with a ligand having affinity for the component, said ligand selected from the group consisting of an antibody to BRD4, an antibody to a Mediator component, and an antibody to H3K27Ac, to form a complex between the cross-linked complex and the ligand;

determining an amount of ligand bound to each enhancer in the cross-linked complex in the cell; and

utilizing the determined amount of ligand bound to each enhancer to identify a super-enhancer,

wherein the identified super-enhancer is bound to at least 10-fold more ligand than the median amount of ligand bound to enhancer within the cell.

2. The method of claim **1**, wherein the component is BRD4 and the ligand is an antibody to BRD4.

3. The method of claim **1**, wherein the component is a Mediator component and the ligand is an antibody to a Mediator component.

4. The method of claim **1**, wherein the component is H3K27Ac and the ligand is an antibody to H3K27Ac.

5. The method of claim **1**, comprising fragmenting the chromosomal nucleic acid of the cross-linked complex prior to the step of determining the amount of ligand bound.

6. The method of claim **5**, comprising fragmenting the chromosomal nucleic acid after forming the complex between the cross-linked complex and the ligand.

7. The method of claim **1**, wherein the cross-linking of the cross-linked complex comprises covalent cross-linking.

8. The method of claim **1**, further comprising identifying a gene associated with said super-enhancer.

9. The method of claim **8**, wherein said associated gene is identified by proximity to the super-enhancer.

10. The method of claim **8**, wherein said associated gene is identified using high throughput chromatin conformation capture data.

11. The method of claim **8**, wherein the gene associated with said super-enhancer is an endogenous gene within the cell.

12. The method of claim 1, wherein the component is endogenous to the cell.

13. The method of claim 1, wherein determining an amount of ligand bound to each enhancer in the cell is achieved by ChiP-Seq.

14. The method of claim 1, wherein at least a portion of the chromosomal nucleic acid is sequenced after contacting the cross-linked complex with the ligand.

15. The method of claim 1, wherein utilizing the determined amount of ligand bound to each enhancer to identify a super-enhancer comprises:

identifying a portion of the chromosomal nucleic acid that is bound to at least 10-fold more ligand than the median amount of ligand bound to enhancers within the cell.

16. The method of claim 1, wherein utilizing the determined amount of ligand bound to each enhancer to identify a super-enhancer comprises:

determining the median amount of ligand bound to enhancers within the cell;

identifying a portion of the chromosomal nucleic acid that is bound to at least 10-fold more ligand than the median as a super-enhancer.

17. A method of identifying a super-enhancer in a sample comprising chromatin, comprising:

obtaining chromatin from a cell wherein said chromatin has been cross-linked such that chromosomal nucleic acid in the chromatin is cross-linked to a component selected from the group consisting of BRD4, a Mediator component and H3K27Ac with which the chromosomal nucleic acid is associated to form a cross-linked complex;

contacting said cross-linked complex with a ligand having affinity for the component, said ligand selected from the group consisting of an antibody to BRD4, an antibody to a Mediator component, and an antibody to H3K27Ac, to form complex between the cross-linked complex and the ligand;

determining an amount of ligand bound to each enhancer in the cross-linked complex in the cell; and

utilizing the determined amount of ligand to each enhancer to identify a super-enhancer, wherein the identified super-enhancer has an amount of ligand bound that is above the point where the slope of the tangent is 1 in a rank-ordered graph of the amount of ligand bound to each of the enhancers in the cell.

18. The method of claim 17, wherein utilizing the determined amount of ligand bound to each enhancer to identify a super-enhancer comprises:

rank ordering the enhancers according to the amount of ligand bound;

identifying, with respect to the amount of ligand bound, the point where the slope of the tangent is 1 which would occur if said rank order were graphed; and

identifying any enhancer that falls above that point as a super-enhancer.

19. The method of claim 17, wherein utilizing the determined amount of ligand bound to each enhancer to identify a super-enhancer comprises:

graphing, in rank order of the amount of ligand bound, the enhancer and the amount of ligand bound to said enhancer;

identifying the point of the graph where the slope of the tangent is 1; and

identifying any enhancer that falls above that point as a super-enhancer.

20. The method of claim 17, wherein the component is BRD4 and the ligand is an antibody to BRD4.

21. The method of claim 17, wherein the component is a Mediator component and the ligand is an antibody to the Mediator component.

22. The method of claim 17, wherein the component is H3K27Ac and the ligand is an antibody to H3K27Ac.

23. The method of claim 17, comprising fragmenting the chromosomal nucleic acid of the cross-linked complex prior to the step of determining the amount of ligand bound.

24. The method of claim 23, comprising fragmenting the chromosomal nucleic acid after forming the complex between the cross-linked complex and the ligand.

25. The method of claim 17, wherein the cross-linking of the cross-linked complex comprises covalent cross-linking.

26. The method of claim 17, further comprising identifying a gene associated with said super-enhancer.

27. The method of claim 26, wherein said associated gene is identified by proximity to the super-enhancer.

28. The method of claim 17, wherein the component is endogenous to the cell.

29. The method of claim 17, wherein determining an amount of ligand bound to each enhancer in the cell is achieved by ChiP-Seq.

30. The method of claim 17, wherein at least a portion of the chromosomal nucleic acid is sequenced after contacting the cross-linked complex with the ligand.

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